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The Genetics of Insulin-Dependent Diabetes

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Introduction

Insulin-dependent diabetes (IDD) is the predominant form of diabetes in children and young adults. Approximately 25% of patients with IDD do not develop symptoms until midlife or later. Over the past decade, it has been recognized that IDD is the result of a slow, indolent process that belies its often abrupt and dramatic clinical onset. A chronic inflammatory infiltrate of the pancreatic islets, consisting of lymphocytes and occasional macrophages and accompanied by loss of some 90% of β cells, is seen in the pancreas of IDD patients at the time of clinical presentation.

It also has become increasingly apparent that IDD is the result of an autoimmune process. At this writing, the first reports of amelioration of IDD in newly diagnosed patients by immunosuppressive therapy have appeared in the literature. However, such therapy may have only limited success when initiated at the time of clinical diagnosis, because irreparable loss of β cells already may have occurred. Thus, there is increased interest in early detection of IDD—by screening populations at risk for circulating islet cell autoantibodies (ICA) as well as by identifying the relevant genetic factors that predispose to IDD.

The search for susceptibility genes is important, since the risk of developing IDD is 20 to 30 times greater for first-degree relatives of IDD probands than for individuals in the general population and more than one-third of identical twins affected by the disease are *concordant* for IDD. In contrast, at least half of such identical twins are *discordant* for IDD despite sharing identical genes at birth. This high rate of discordance argues that environmental agents are needed to initiate the pathogenic process in those who are genetically predisposed. However, convincing evidence that any one agent plays a major role in this regard is lacking.

One Gene or Several?

Several European groups have reported that more males than females are affected by IDD, as indicated by a male:female ratio of 1.2:1. Although this observation has not been clearly supported by American studies, our own unselected group of patients in Florida has a 10% excess of male patients, with the greatest excess seen among patients in two distinct age groups: those with IDD onset before 6 years of age and those with late-adolescent onset. Thus, a consensus view would hold that male gender represents a definite, but minor, genetic influence on disease susceptibility.

Thyrogastric autoimmunity genes are another genetic system of interest in patients with IDD. For example, thyroid microsomal and gastric parietal cell antibodies occur in IDD patients at a rate four to six times that of nondiabetic controls matched for age, sex, and race. Although the incidence of thyroid and gastric autoantibodies increases dramatically with age in the general population, such incidence in children with IDD is equivalent to that in the geriatric general population. A greater prevalence of these autoantibodies are also found in nondiabetic parents of children with IDD. If the dominantly inherited tendency for production of thyroid and gastric autoimmunities is separate from the major genetic influence for IDD susceptibility, then thyrogastric autoimmunity must predispose to IDD in its own right.

Indeed, data gathered by Riley et al have confirmed a report by Cudworth et al that thyroid and gastric autoantibodies in families with IDD do not segregate with human leukocyte antigen (HLA) haplotypes. In contrast, HLA haplotypes do segregate with the occurrence of IDD in multiplex pedigrees. Thus, thyrogastric autoantibody genes are distinct from those for IDD and may also convey genetic susceptibility to IDD. IgG heavy-chain allotypes may also influence the occurrence of IDD, as documented in Graves' disease.

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HLA-Associated Inherited Susceptibility

The above factors notwithstanding, the major genetic factor in the inherited susceptibility to IDD is that associated with the HLA system. A primary association of IDD with HLA-DR3 and HLA-DR4 has been reported by a large number of investigators. In our experience, only 5% of children and young adults with IDD lack one or both of these antigens, whereas approximately 40% of patients have both antigens—ie, are DR3/DR4 heterozygotes. However, HLA typing of random individuals in the general population provides little information that would help to identify individuals at risk for development of IDD.

As shown in the table, the absolute risk for developing IDD, as determined in collaboration with Rotter et al, is no higher than 1 in 40 for the highest-risk HLA phenotype, the DR3/DR4 heterozygote. HLA-DR2 and HLA-DR5 are infrequently found in patients with IDD, and are thus associated with low absolute risk (about 1 in 2,500) for IDD.

When the absolute risk for IDD is applied to family members of a diabetic proband, the sharing of HLA haplotypes containing the IDD-associated DR3 or DR4 alleles conveys greatly increased risk for IDD. For an HLA-identical sibling of a diabetic proband, the absolute risk for IDD is approximately 1 in 7; it rises to 1 in 4 if the shared haplotypes contain both DR3 and DR4 antigens. This risk is ten times greater than that for persons in the general population who are also DR3/DR4 heterozygotes. This implies that HLA haplotypes containing DR3 or DR4 associated with observed IDD are different from those seen in the nondiabetic general population. This conclusion is reinforced by the fact that the frequencies of particular alleles at other polymorphic loci on chromosome six are shown to be in linkage disequilibrium with DR3 and DR4. Thus, A2, Cw3, and Bw62 are in linkage disequilibrium with DR4, since these particular alleles of the A, C, and B loci are found to be associated more frequently with DR4 than would be expected. This extended haplotype is present at an increased rate in IDD patients, and is much less common among DR4-positive individuals in the general population.

Absolute Risks for IDD for Caucasian Persons of Various HLA Phenotypes and Genotypes (Based on an IDD Prevalence Rate of 1 in 500)

HLA Phenotype		HLA Genotype	
DR1	1 in 1,000	DR3/DR3	1 in 125
DR2	1 in 2,500	DR3/DRX	1 in 500
DR3	1 in 185	DR4/DR4	1 in 147
DR4	1 in 208	DR4/DRX	1 in 476
DR5	1 in 2,500	DR3/DR4	1 in 42
DR6	1 in 1,429	DRX/DRX	1 in 5,565
DR7	1 in 1,250		
DR8	1 in 556		
DR9	1 in 345		

In addition, this haplotype and other extended haplotypes associated with IDD may involve particular complement allotypes. In our observations of more than 1,000 patients with IDD, we have found a distinct excess of DR1-positive patients among the 5% of IDD patients who lack both DR3 and DR4 antigens. This is consistent with the idea that the HLA-associated IDD susceptibility gene or genes are separate from DR, but are in linkage with DR3, DR4, and—in some instances—DR1. [Autoimmune Addison's disease occurring as part of the Type II autoimmune polyglandular syndrome (Schmidt's syndrome) is also associated with DR3 and DR4 antigens, although it appears to be unrelated to HLA when it occurs as part of Type I autoimmune polyglandular syndrome (moniliasis/hypoparathyroidism).]

Predictive Value of HLA Typing

HLA typing of individuals at random gives little information to predict latent IDD, although certain HLA-DR phenotypes, such as DR2/DR2, DR2/DR6, DR2/DR5, DR5/DR5, and DR5/DR6, are very rarely associated with IDD. However, HLA typing in pedigrees affected by IDD can be valuable in identifying at-risk individuals according to the number of haplotypes shared with the diabetic proband. Since ICA has been shown to be a useful marker for IDD, it was of interest to learn whether the occurrence of ICA is restricted to those with inherited susceptibility for IDD. In our studies, such was the case. ICA among siblings of diabetic probands was limited to those sharing HLA haplotypes, being most common among the HLA-identical siblings and having an occurrence rate similar to that of IDD. Furthermore, ICA in the general population was restricted to those with HLA phenotypes containing DR3 and/or DR4, the IDD risk alleles. Therefore, in persons with ICA, HLA typing does not significantly add to the predictive value of this marker.

Male Segregation Bias and Extended HLA Haplotypes

Warram recognized that fathers with IDD were some five times more likely than mothers with IDD to produce children of either sex with IDD. This remarkable finding has been confirmed by many others. In our studies, Vadheim has shown that HLA haplotypes bearing DR4 are preferentially passed on to offspring from fathers of children with IDD; this was not the case for the mothers. This implies that the "diabetes-prone" DR4-bearing HLA haplotypes from fathers might enhance the chance of conception or, alternatively, convey some survival advantage to the fetus in utero.

Alper has shown that extended haplotypes involving HLA-A, C, B, complement Bf, C2 and C4, and HLA-D loci, and even the more-centromeric erythrocyte glycoxylase locus (GLO-1), participate in the HLA-associated susceptibility to IDD. Such extended haplotypes in linkage disequilibrium may be unusually resistant to recombinant events. However, the reasons for this and the implications for genetic susceptibility to IDD are not yet clear.

Molecular Genetics of the HLA Complex

The class II antigens of the major histocompatibility complex are heterodimeric cell surface glycoproteins involving relatively conserved α chains and more variable β chains. In man, the three major class II antigens have been identified as DR, DQ, and DP. The DQ loci are closely situated to DR; the DP loci are more-centromeric loci on the short arm of chromosome six (Figure 1). The DR cluster contains one, possibly two, α and three β genes. One of these β genes may be a pseudogene and is therefore not expressed. There are two α- and two β-coding DQ genes and two α and two β DP genes.

Whereas the DR antigens (DR1-10 and DRw52/w53) and the DQ antigens (DQw1-3) can be determined by serological typing (using maternal-derived alloantisera), DP typing can be performed only by using mixed lymphocyte reactions or molecular biologic techniques. HLA is currently being studied at the genomic level through restriction fragment length polymorphism (RFLP) analysis of all of the HLA-D genes. This technique involves harvesting DNA from peripheral blood or from lymphoblastoid cell lines established by transformation of peripheral B lymphocytes with Epstein-Barr virus. The genomic DNA is digested with restriction endonucleases, which cleave the DNA at sites of specific base sequences; the resulting fragments are separated by size using agarose gel electrophoresis. Using the Southern blotting method, the DNA is transferred to nylon filters, which are then hybridized with ^{32}P-labeled DNA probes (cDNA or genomic types) to identify the genes of interest. Using such an approach with DRα and DRβ probes, Erhlich has identified RFLPs that differentiate DR3 haplotypes involving A1 1B8 DR3 haplotypes in diabetes-prone individuals from other haplotypes.

Other investigators, including those in our laboratory, have concentrated on DQβ and DQα RFLPs associated with DR4. To date, DR and DQ RFLP analysis has identified HLA haplotypes that are more likely to convey inherited susceptibility to IDD than could be determined by serological typing for DR antigens alone (Figure 2). An absolute relationship between specific RFLPs of class II genes and IDD has not yet been found. However, as reported by Owerback et al, a 3.7 kb RFLP, seen when genomic DNA is digested with the restriction endonuclease Bam H1 and probed with a DQβ cDNA, is infrequently found in DNA from IDD patients. To date, we have found this fragment to be absent on both DR4-bearing chromosomes in DR4/DR4 IDD patients. This implies that only DR4-bearing haplotypes that lack the 3.7 kb DQβ RFLP convey susceptibility to IDD. The site and nature of the HLA-associated IDD susceptibility gene or genes has yet to be identified.

The HLA Region on the Short Arm of Chromosome Six

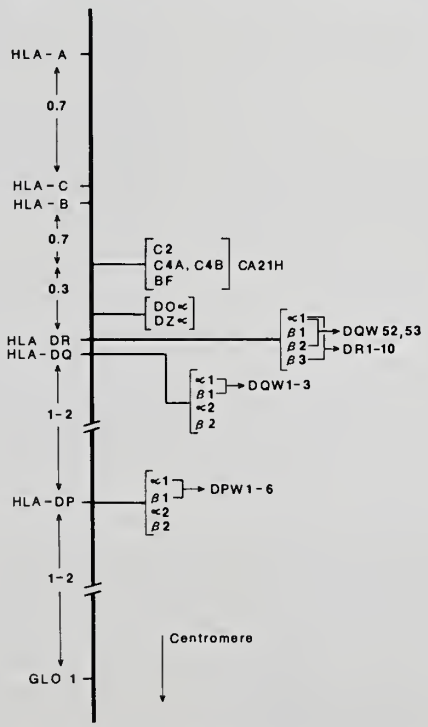

Figure 1. The map of the greater HLA genomic region is depicted. HLA-D molecules are heterodimers of α and β chains. Transcomplementation products combining an α chain coded by one chromosome with a β chain of the same antigen class from the other chromosome—as well as combinations of α and β chains of a single chromosome—occur at each locus.

Insulin Gene Polymorphism

The 5′ flanking region of the human insulin gene is extremely polymorphic because of variable numbers of tandem repeats of 14-15 base pair oligonucleotide sequences. Although three classes of alleles have been defined, there is great variation within each class and thus there are many unique alleles. Interest in this region grew rapidly once its polymorphic nature was identified by Bell, because diabetes theoretically could result from defective regulation of insulin gene transcription. To date, non-IDD, atherosclerosis, hyperlipidemia, and IDD have all been reported to have associations with particular haplotypes, although these issues remain controversial. In our laboratory, Winter has identified a similar, but much abbreviated, polymorphism in the 5′ flanking region of the insulin I gene in rats. Unlike man, rodents have two insulin genes. The insulin II gene is analogous to that in man. The spontaneously diabetic Biobreeding rat (which inherits diabetes as a recessive trait, according to Colle) has both IA and IB RFLP alleles in roughly equal numbers. However, IDD is not related to either allele.

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Summary

Despite a growing body of literature on the genetics of IDD, the inherited predisposition to the disease cannot yet be identified with any great precision. There is at least one major susceptibility gene within the HLA-D region of chromosome six that is still to be mapped. There are other minor genes—those involving thyrogastric autoimmunities, male gender, insulin gene polymorphisms, and Ig heavy and light chain allotypes—that may affect the outcome. Immunoregulatory genes of the T-lymphocyte receptor will soon be subjected to study. In addition to these genetic influences, there is evidence that an environmental trigger also may be necessary for IDD to develop. It is anticipated that genetic and environmental interactions underlying IDD will soon be unraveled and translated into methods that will prevent disease.

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References will be provided upon request to Dr. Blizzard.

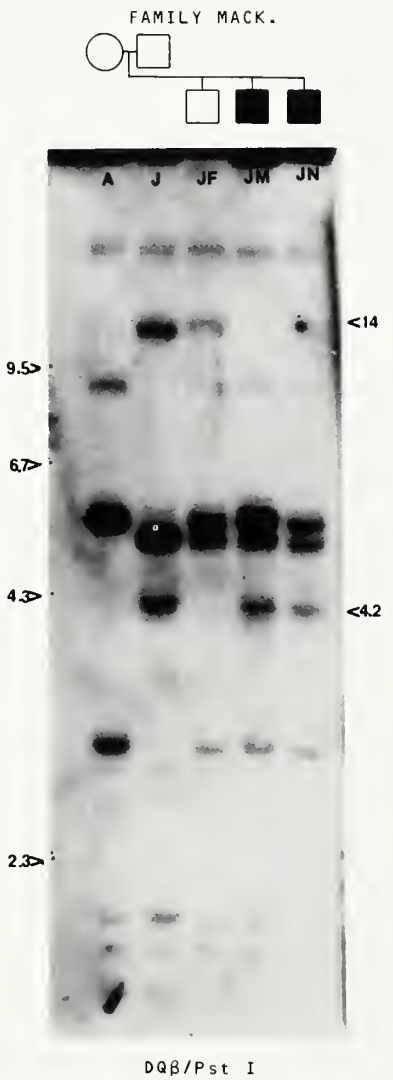

Figure 2. The pedigree includes three sons (two with IDD as identified by the black squares) and both parents. Mother is an HLA-DR3 homozygote; father, a DR4 homozygote. A Southern blot of a Pst 1 endonuclease digestion of genomic DNA, after hybridization with a DQβ, cDNA probe, is shown. The center of the blot shows homozygous bands of approximately 5.8 and 5.4 kb in the parents, with combinations of both bands in all three DR3/DR4 heterozygous children. The nondiabetic son inherited the 14 kb DQβ RFLP allele in association with one DR4 haplotype, while the two diabetic sons have a 4.2 kb DQβ RFLP allele from the father, marking the IDD-associated DR4 haplotype in this family.

In Future Issues

Hypophosphatemic Hyperphosphaturic Rickets: An Update by Harold Harrison, M.D.

Fetal Growth and Growth Factors by Joseph D'Ercole, M.D.

The Relationship Between Endurance-Type Training and Adolescent Development by Alan D. Rogol, M.D., Ph.D

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Diabetes Control and Growth Hormone: New Insights

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Type I diabetes is characterized by a variety of metabolic and hormonal abnormalities in addition to hyperglycemia. Elevations in plasma growth hormone (GH) levels frequently have been observed in Type I diabetics, but the mechanisms by which these elevations occur have been difficult to establish. Arguments that GH hypersecretion plays a role in perpetuating the metabolic derangements of diabetes have waxed and waned in popularity. The introduction of intensive insulin treatment regimens and other new in vivo techniques has provided considerable information about the complex interrelationships between metabolic control of diabetes and GH secretion and action. Studies in these areas have produced results that have implications concerning GH regulation in nondiabetic subjects as well.

Influence of Metabolic Control on GH Secretion

Hansen was the first to show reversibility of GH hypersecretion in response to exercise by intensive treatment with multiple injections of insulin. Similarly, basal and post-exercise GH concentrations have been restored to normal following continuous subcutaneous insulin infusion (CSII) treatment, which also normalized the elevated mean 24-hour GH concentrations.

Several studies have tried to ascertain the level at which diabetes affects central regulation of GH secretion. Although there is little evidence to suggest that metabolic control of diabetes directly influences the pituitary, indirect effects have been demonstrated. For example, the pituitary response to stimulation with GH-releasing factor is normal when diabetics are hyperglycemic, but not when they have normal plasma glucose concentrations. On the other hand, hypersecretion of GH has been observed in poorly controlled patients in response to several stimuli, such as arginine, L-dopa, and clonidine, that are thought to act through the hypothalamus. Furthermore, improved control with CSII reduces to normal the GH response to clonidine (an α_2-adrenergic agonist), but does not affect the pituitary response to GH-releasing factor.

Deranged hypothalamic regulation of GH secretion in diabetes may be more selective than the above might indicate. For example, Simonson et al used a modification of the insulin clamp procedure to study the effects of improved metabolic control with CSII on the GH response to hypoglycemia, the classical hypothalamic stimulus. Surprisingly, the GH response to a lowering of plasma glucose from 90 to 50 mg/dL was normal when the patients were poorly controlled and suppressed to subnormal values after eight months on CSII.

Influence of Metabolic Control on Insulin-Like Growth Factors

Because GH levels tend to be increased rather than reduced in poorly controlled diabetes, investigators began to look for possible diabetes-induced defects in other circulating growth factors, particularly insulin-like growth factor I (IGF-I). Early studies in diabetics receiving conventional treatment yielded contradictory results, with normal, elevated, and reduced levels of IGF-I being reported. These discrepancies may have resulted from differences in methodologies (eg, bioassay *v* radioimmunoassay), variability in the level of diabetic control, and failure to account for the increase in IGF-I that accompanies normal pubertal development. Thus, it is noteworthy that Blethen et al recently found a negative correlation between glycosylated hemoglobin and age-adjusted IGF-I values in conventionally treated adolescents with diabetes.

Recent studies of CSII have clearly demonstrated that inadequate insulin replacement results in a defect in IGF-I synthesis. Not only were IGF-I levels reduced in poorly controlled diabetic patients, as compared with age- and sex-matched normal controls, but IGF-I levels increased by 25% after only one week of CSII despite a fall in GH values. A further increase in IGF-I concentrations is observed with more prolonged improvement in metabolic control. Lanes et al showed that IGF-I response to exogenous GH administration was blunted in poorly controlled diabetics when compared to the response in relatively well-controlled subjects.

In contrast, metabolic control of diabetes appears to have little effect on IGF-II values. IGF-II levels have been reported by Amiel et al to be normal in both adults and adolescents with diabetes, and no change in mean values was noted after CSII. However, in the latter study, four of 19 patients with very low IGF-I levels also had depressed IGF-II concentrations (which returned to normal with improved metabolic control). Thus, except in the most severely affected patients, compensatory increases in GH appear to be sufficient to maintain adequate IGF-II production.

Influence of GH on Metabolic Control

Hypersecretion of GH may also help perpetuate the metabolic derangements of diabetes to a much greater extent than is generally appreciated. In a recent study, Press et al administered exogenous GH as hourly intravenous pulses to a group of diabetics who were optimally controlled with CSII. Prior to GH administration, mean 24-hour plasma glucose and GH levels were within the normal range. However, when serum GH was raised to levels seen in poorly controlled patients, a progressive rise in plasma glucose was observed. Surprisingly, the hyperglycemia was primarily due to a marked stimulation of hepatic glucose production. GH is usually thought to increase glucose concentrations in diabetics, as in non-

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diabetics, by inhibiting glucose uptake in peripheral insulin-sensitive tissues.

The adverse metabolic effects of GH demonstrated in this study were not confined to plasma glucose levels. Levels of circulating fatty acids, ketones, and branched-chain amino acids were also increased. Therefore, GH elevations can themselves produce the entire spectrum of abnormalities associated with poor diabetic control, despite previously optimized insulin treatment. It follows that some of the metabolic benefits of more intensive insulin regimens may be derived from GH-lowering effects.

The "dawn" phenomenon is a significant cause of glycemic lability in insulin-dependent diabetics. Plasma glucose and basal insulin requirements vary considerably during the night. Both reach a nadir between 2 and 4 AM and then rise together as daylight approaches. It was originally thought that these changes reflected diurnal fluctuations in plasma cortisol. However, a delayed anti-insulin effect exerted by the early nocturnal surges of GH may be a more likely explanation; however, not all investigators agree with this concept.

Diabetes in adolescents is particularly difficult to control. Although control problems are usually attributed to psychosocial and dietary factors, the hormonal changes of puberty might also play a role. If such is the case, the puberty-associated rise in GH would be expected to be a contributing factor. In a recent study, insulin sensitivity was determined in preadolescents and adolescents, with and without diabetes, using the euglycemic, hyperinsulinemic insulin clamp technique. It should be noted that insulin-mediated glucose metabolism was reduced in diabetic patients *and* healthy children with the onset of puberty. Furthermore, the degree of insulin resistance was directly correlated with mean 24-hour GH concentrations.

Influence of Metabolic Control on Linear Growth

The observation that most conventionally treated diabetic children appear to be growing at a normal rate despite a host of metabolic and hormonal derangements is a testament to compensatory mechanisms that help sustain growth. However, Tattersall and Pyke found that patients who had developed diabetes before puberty were shorter as adults than their nondiabetic identical twins. The view that diabetic children may not be achieving their full growth potential is supported by studies that employ intensive treatment. Adolescents with diabetes—even those with normal stature and apparently normal growth rates on conventional therapy—show a sharp increase in growth velocity during treatment with either CSII or multiple injections.

Although it is attractive to speculate that the increased growth seen in well-controlled adolescents is the result of optimized therapy, such an interpretation is limited by difficulties in assessing growth velocity changes during puberty. To examine this further, we used a clonal stem cell assay for proliferation of erythroid progenitors (burst forming units-erythroid [BFU-E]) to determine the effect of CSII on cellular growth in vitro. This assay system has been useful in assessing other causes of growth retardation. Blood was obtained from eight diabetic patients before and after one week of CSII. Numbers of BFU-E–derived colonies were not different from normal during conventional treatment, but increased sharply after one week of CSII. These changes in the in vitro cellular growth are strikingly similar to the long-term effects of intensive treatment on linear growth in adolescents. The ability to detect very rapid changes in cellular growth with the BFU-E assay illustrates the primary importance of improvements in fuel metabolism.

Summary

Viewed together, the observations presented in this article have important clinical implications. In the poorly controlled diabetic patient, a vicious cycle—whereby hypersecretion of GH acts as a compensatory response to a reduction in IGF-I—may become established. The associated rise in GH causes a worsening of metabolic control, further impairing the somatotropic action of GH. The efficacy of intensive treatment may be due to an interruption of this cycle that lowers circulating GH concentrations. Normalization of hormonal milieu and improvement in growth rates are associated with good diabetic control. Consequently, physicians should make every reasonable effort to normalize glucose metabolism in juvenile diabetic patients. For those interested in reading further about the role of insulin in growth, an abstract entitled "Insulin as a Growth Factor" appears on this page.

References are available upon request to Dr. Blizzard.

Insulin as a Growth Factor

In their review, Hill and Milner discuss various aspects of insulin's capability to promote growth (see related article by Tamborlane and Amiel on page 5 of this issue).

Insulin does influence in vivo growth. Although growth hormone (GH) is largely responsible for the rise and fall of insulin-like growth factor I (IGF-I), other factors also play a role, including insulin itself. Disordered growth consequent to insulin dysfunction is frequently associated with a parallel change in circulating levels of IGF, suggesting a direct or indirect modulation of IGF production by insulin.

The similarity in structure between the IGFs and insulin allows low affinity binding between insulin and IGF receptors and vice versa. There are three individual receptors: one for the action of insulin, one for IGF-I, and one for IGF-II. When stimulated, each of these is capable of inducing mitogenesis, and each is capable of accepting the other two growth factors if it is not already "occupied" by its primary growth factor. The authors emphasize that insulin, therefore, may exert direct mitogenic action—and consequently, growth—through either insulin or IGF recep-

tors. Their paper reviews experimental evidence (and draws some clinical parallels) in support of the concept that insulin has both direct and indirect roles in the control of normal body growth.

Insulin, in addition to its other actions, acts as a necessary mediator of IGF-I generation. Several mechanisms are possible. In rats, a decrease in the amplitude of GH peaks has been observed in the absence of insulin. However, GH administration does not stimulate IGF-I levels. Another probable mechanism is via a direct modulation of IGF release from the liver to other tissues. Insulin also may mediate IGF release indirectly by altering the GH/IGF axis, since there appears to be a severe reduction in the number of GH receptors in the liver of ketotic diabetic rats. Interestingly, in other studies cited by the authors, serum levels of IGF-I have increased and growth of the tibial epiphyses has occurred in hypophysectomized rats (GH deficient) whose pancreases were stimulated to release insulin by the administration of a sulfonylurea. This clear demonstration that insulin could modulate IGF release independent of GH indicates what may be an important mechanism for the growth retardation seen in experimental diabetes.

Another example of the important role of insulin in growth is the presence of inhibitory factors that oppose the action of both IGF and insulin in animals with chemically induced diabetes. As a consequence, tissue anabolism is impaired. The inhibitor is not specific to insulin and insulin-like peptides, but has a general depressive action on all aspects of cartilage metabolism. In the diabetic rat, this factor appears to originate in the liver.

Insulin dysfunction and postnatal and prenatal growth disorders are considered by the authors. Diabetes in childhood is the most common clinical example of disturbed growth due to abnormal insulin secretion. Growth may be subnormal for months before the diabetes is clinically manifested, and treatment is closely linked to the quality of diabetic control. Mauriac syndrome (in which the child is short, obese, and has a large liver with fatty infiltrations) results from excess dietary carbohydrate coupled with excess insulin and brittle control. The authors cite a study by Winter et al of a 7-year-old with normal GH release in response to insulin hypoglycemia but with very low IGF-I values. However, IGF rose on each of two occasions when metabolic control was improved. Winter et al speculated that a block in the GH/IGF axis existed in poorly controlled diabetics since IGF-I did not rise with GH administration in this patient during periods of poor control.

Other studies from the literature cast further light on the relationship of diabetes and growth. One found that adult diabetics are consistently shorter than their identical but nondiabetic twins. Another found an inverse relationship between IGF-I and HbA1c in 40 diabetic children. Still another series found that GH values were higher than expected in diabetics with normal IGF-I values, suggesting a blunted response of IGF to GH concentrations.

The authors cite a study conducted by Rudolf, Tamborlane et al on the growth potential of children with relatively well-controlled diabetes (they measured growth velocity in nine insulin-dependent children before and after six months of intensive insulin treatment via pumps or multiple injections). During conventional therapy (injections of insulin once or twice daily), the mean growth velocity was 5.3 cm/yr, a rate within the low normal range, despite evidence of intermittent hyperglycemia. After a period of intensive management, in which the overall dose of insulin was not increased, mean plasma glucose fell from 270 to 105 mg/dl, and glycosylated hemoglobin fell from 12.4% to 8.4%; the mean growth velocity increased sharply to 9.4 cm/yr as the serum IGF-I level doubled. The rate of skeletal maturation did not increase. The conclusion was that improved metabolic control, even for children who were not obviously short, could substantially increase adult height potential.

A follow-up study examined the circulating IGF-I and II levels in diabetic children by specific radioimmunoassays. During conventional therapy, IGF-I was lower, but IGF-II was generally unaltered in 19 insulin-dependent diabetics as compared with nondiabetic controls. Following one week of intensive insulin therapy, IGF-I values increased by 25% despite a decrease in the mean 24-hour levels of GH. Circulating IGF-II did not alter during intensive therapy. This study provided further evidence that the normal control of IGF-I by GH is disrupted in poorly controlled diabetics, and that this can be partially corrected by improved metabolic control. In contrast, endogenous hyperinsulinemia in childhood is not associated with a serious disturbance of growth. Blethen et al described seven children, less than 3 years of age, who had severe fasting hypoglycemia due to hyperinsulinemia. Neither IGF-I nor IGF-II differed from the values for age-matched control children.

It has been reported that cultured skin fibroblasts from patients with insulin– or non–insulin-dependent diabetes show increased sensitivity to insulin. Indeed, cells from diabetic patients were more sensitive to insulin than those from nondiabetics with respect to collagen synthesis. This may shed some light on the etiology of macroangiopathy in diabetes, since collagen comprises more than half the total protein present in human atherosclerotic plaques. Fibrous deposition in diabetics may originate from smooth muscle cells that proliferate in the subintima and deposit forms of collagen that are chemically distinct from those found in normal subjects. Insulin-dependent diabetics with atherosclerosis were also found to have higher circulating insulin levels than those without diabetes.

Leprechaunism, a rare dwarfing phenomenon seen in the neonate, is characterized by insulin resistance, poor stores of subcutaneous tissue, and intrauterine growth retardation. It is associated with various defects of the insulin receptor, including the absence of insulin receptors in some patients and postreceptor defects in others. Hill and Milner emphasize that insulin resistance at either a receptor or postreceptor site is seldom isolated from a resistance to the biologic actions of the other peptide growth factors, resulting in intracellular malnutrition, impaired growth, and, in many cases, early death.

In utero defects of insulin secretion and utilization are seen in syndromes other than leprechaunism. For example, the infant of the diabetic mother whose glucose me-

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tabolism is poorly controlled is obese and often has visceromegaly. Although insulin is present in the human fetal pancreas as early as the tenth week of gestation, insulin release remains insensitive to glucose until the gestational age of approximately 28 weeks, at which time the preadipocyte matures into an insulin-sensitive cell capable of accumulating lipid. Most of the excess weight seen in the infant of a diabetic mother is fat accumulated during the last trimester of pregnancy. The less dramatic but unequivocal increase in somatic growth that occurs concurrently suggests that insulin has an additional direct or indirect role in protein synthesis and cellular proliferation. Enhanced fetal somatic development has been described in infants with nesidioblastosis or the Beckwith-Wiedemann syndrome, each of which is associated with hypersecretion of insulin. Conversely, in transient neonatal diabetes and in pancreatic agenesis, the newborn is characteristically small-for-dates, has poor muscle bulk, and has virtually no adipose tissue.

There are several pathways by which insulin can act as a fetal growth factor. First, it may alter cellular nutrition by increasing nutrient uptake and utilization. Second, insulin may exert a direct anabolic action via either the insulin or the IGF-I receptor. Third, insulin may modulate the release of IGF or other growth factors from fetal tissues. Although the authors found no direct mitogenic action of insulin on human fetal fibroblasts or myoblasts obtained from fetuses at less than 20 weeks' gestation, it is conceivable that insulin may exert a direct growth-promoting action during later fetal development. Since the insulin receptor population may be abnormally elevated in some tissues of infants born to diabetic mothers, one can postulate that this, coupled with hyperinsulinemia, may result in a direct, pathophysiologic stimulation of human fetal somatic and skeletal growth.

Based on the experimental and clinical data regarding the endocrinology of the overgrowth seen in infants of diabetic mothers, two deductions seem reasonable: (1) Body length is increased slightly, if at all, even in the presence of extremely high insulin levels and a raised IGF level, suggesting that normal fetal growth is taking place close to its maximum potential; (2) modest hyperinsulinemia can result in organomegaly and obesity despite normal circulating IGF values. These effects appear to be due to either direct anabolic and lipogenic actions of insulin or to another, as yet unidentified, mediator.

The parallel changes in serum insulin and IGF levels, especially those seen in fetal growth retardation, suggest that some of the anabolic actions of insulin in utero may be mediated by a change in IGF release. In the fetuses of many species, including humans, body growth and circulating IGF levels do not depend on the presence of pituitary GH; in fact, growth persists after experimental decapitation in the rabbit or hypophysectomy in the sheep. The immaturity of the GH/IGF axis may be related to the observations that somatotropic receptors do not appear in the liver of the sheep or rat until after birth. Any prenatal regulation of IGF release by insulin is therefore unlikely to be mediated by changes in GH secretion or by changes in the nature of the GH receptors.

The authors conclude that insulin functions as a growth factor at the cellular level and within the whole body. Yet, for many tissues, insulin does not appear to be the major circulating anabolic agent. The secondary position of insulin in the endocrine control of mammalian growth may derive from a diversification of biological function among the insulin-related family of molecules. In most mammalian species, the IGFs, and predominantly IGF-I, have evolved as the more potent mitogenic peptides while insulin fulfills a more acute metabolic function. Similarly, the IGF-I receptor, rather than the related insulin receptor, has become the most utilized initiator of a positive pleiotypic response. However, this is a gross generalization and, for particular tissues, such as the liver, insulin still may act as a potent mitogen via the insulin receptor. In addition, insulin may continue to exert control of the development of skeletal tissues, in association with intracellular nutrition, by regulating IGF release. Pathophysiologically, insulin may assume the role of a major growth-promoting agent if overproduction is associated with extensive binding to the IGF-I receptor, as may occur in the infant of the diabetic mother.

Hill DJ, Milner RDG: *Pediatr Res* 1985;19:879.

Editor's comment—*The authors present an outstanding and complete review of insulin as a growth factor. This abstract discusses only a minor portion of the material covered, and the editor encourages all readers to review the article in its entirety.*

The Short Child With Subnormal Plasma Somatomedin-C (Sm-C)

The somatomedin-C (Sm-C), or insulin-like growth factor I (IGF-I), level is being used as a screening test for growth hormone deficiency (GHD). To evaluate its diagnostic value, the authors designed a protocol to evaluate: (1) the statistical tolerance limits for Sm-C in children of normal height between 7 and 10 years of age; (2) the prevalence of subnormal Sm-C in children of the same age who are below the third percentile in height; (3) the prevalence of GHD in children with low Sm-C levels; and (4) the comparison of linear growth responses to hGH treatment between GHD children and hyposomatomedinemic, non-GHD short children.

Single Sm-C determinations were reported to be of limited value in diagnosing GHD. Only with an average of four determinations (taken at six-week intervals) in seven GHD children could all seven be said to have an average Sm-C level below the 95% lower limit of the tolerance intervals, as based on the mean of one, two, three, or four determinations.

In 97 short non-GHD patients whose Sm-C levels were measured

four times and then averaged, 45% (or 44 children) were below the 2.5 percentile established for normal children. Of these 44 children, who were considered to be hyposomatomedinemic, 12 were classified as GHD, seven as partially GHD, 20 as non-GHD, and five as intermediate in their responses or non-classifiable, as determined by the usual pharmacologic testing. Therefore, 19 of the 44 (or 43% of the hyposomatomedinemic children) and 19 (or 20%) of the 97 children with heights below the third percentile had some degree of diagnosable GHD. The anthropometric measurements and skeletal ages in relation to the chronological ages were identical in the 19 GHD and 20 non-GHD patients, as were the levels of Sm-C. Consequently, the authors deduce that approximately 20% of short children referred to them will be GHD.

Therapy with hGH was given to the GHD and non-GHD hyposomatomedinemic children for six-month alternating periods. During each period, one of four logarithmic dosages were admininstered: 0.16, 0.26, 0.43, or 0.70 U/kg/wk were given in equally divided doses Monday, Wednesday, and Friday at 10 PM. The results are shown in the table.

The authors report that the two intermediate doses produced significantly different growth rates in the two treatment groups. However, the largest dose produced comparable growth rates in both groups.

The authors speculate that there are several possible explanations for the low Sm-C determinations in the short non-GHD children, including: (1) a relationship to the delayed skeletal maturation, since Sm-C levels increase with age; (2) failure of nocturnal secretion of hGH; (3) impaired production of Sm-C; (4) a bioinactive GH; and (5) an altered Sm-C binding system.

The authors also speculate that non-GHD children will respond to GH therapy in many instances. At conventional GH doses (up to 0.43 U/kg/wk), the magnitude of the response seen in such children was less than 60% as great as that of their GHD counterparts; however, at the dose of 0.70 U/kg/wk, the responses of the four GHD children and the three non-GHD children were comparable.

Rudman D, Kutner MH, Chawla RK: *Pediatr Res* 1985;19:975.

Dose hGH (U/kg/wk)	Increase in growth velocity	
	GHD	Non-GHD
0.16	4.4 ± 0.7 (n = 8)	0.2 (n = 1)
0.26	7.4 ± 1.2 (n = 9)	3.2 ± 0.7 (n = 16)
0.43	8.7 ± 0.9 (n = 12)	5.4 ± 0.7 (n = 12)
0.70	8.3 ± 1.1 (n = 4)	7.3 ± 2.0 (n = 3)

Editor's comment—*Although the authors state that four Sm-C determinations were necessary to unequivocally diagnose GHD in the seven GHD patients studied for this purpose, a review of the data of the 28 determinations made in these patients reveals that only two determinations were greater than 0.30 U/ml. Therefore, 26 of the 28 determinations yielded values that were certainly compatible with GHD, although not diagnostic thereof. In addition, the finding of a low Sm-C (<0.30 U/ml) by averaging four determinations in a short child would be associated with the diagnosis of GHD in only 40% of cases (19 of 44 children in this series). Therefore, the practicing physician can still effectively utilize a single Sm-C determination in evaluating the possibility of GHD. It should be noted, however, that the Sm-C determination is only one facet of the diagnostic evaluation of a short child.*

Behavioral Problems and Social Competence in Girls With True Precocious Puberty (TPP)

The authors evaluated 33 girls between 6 and 11 years of age with true precocious puberty (TPP) of various etiologies. At the time of presentation, 55% were above the 95th percentile for height-for-age; bone age was advanced by two to five years in all subjects. Before treatment, the parent(s) completed a 120-item child behavior checklist, from which a child behavior profile was generated. It consisted of three social competence scales, nine behavior problem scales, and two second-order factors (internalizing or externalizing scales). The personality profiles were compared with those of matched controls, and appropriate statistical data were extracted.

Many, but not all, of the girls were reported to have behavior problems. For example, 27% had a total behavior problem score at or above the 98th percentile for normals and many scored significantly higher than controls in all of the internalizing factors—eg, depression, social withdrawal (45% >97th percentile), somatic complaints (30%), and schizoid/obsessive traits. The incidence of hyperactivity and aggressiveness was significantly higher in TPP patients than in controls. The authors considered whether all these increases could be related to the expected changes of behavior that occur in adolescence and determined that such was not the case.

Other behavioral traits that were frequently observed in these girls included clinging to adults, feelings of worthlessness, sulking, fatigue, strange or unpredictable behavior, inability to sit still, daydreaming, crying, teasing, temper tantrums, and whining. They also tended to sleep less than most children.

Overall, the girls with TPP could be described as troubled, depressed, aggressive, socially withdrawn, and moody. The authors emphasize, however, that to view these children as psychiatrically disturbed and/or in need of psychiatric treatment is to misinterpret the findings. The behavioral "breakdown" reported may reflect the

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True Precocious Puberty

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mechanisms for maintaining homeostasis in an abnormal environment. These children need to cope with an age-appearance disparity that modifies the response of their social milieu. Adults expect children to perform tasks that are commensurate with height age. Consequently, these children may have an abnormal body image, lack self-confidence, or prefer to be by themselves. Their social withdrawal may well be related to the disparity of age and appearance and expected social behavior.

Sonis WA, Comite F, Blue J, et al: *J Peds* 1985;106:156.

Editor's comment—*In their summary, the authors emphasize that a majority of the girls in their series did not have behavioral problems, although a significant and large minority did have a dysphoric and stressful adjustment.*

*Relevant to this report is one by Ehrhardt et al (*J Am Acad Child Psychiatry *1984;23:1), entitled "Idiopathic Precocious Puberty in Girls: Psychiatric Follow-Up in Adolescence." This was a systematic, controlled study of psychopathology in 16 adolescent girls between 12 and 13 years of age, significantly older than the patients studied by Sonis et al. The average height (160.7 cm) was below the average height (166.4 cm) of the controls, as would be expected in females with a history of sexual precocity. Patients and controls were similar regarding various aspects of self-image, except for marginal differences in morals and sexual attitudes. The patients had a somewhat less positive attitude toward sexuality, rating having a boyfriend as far less important than did the controls. There were also marginal differences in intellectual and school status, with decreased popularity and less anxiety being associated with the patients. Conduct problems, antisocial behavior, inadequacy or immaturity, and socialized delinquency were marginally increased in the patients.*

It is important to note that both sets of authors stressed that an increased incidence of definitive psychiatric disorders was not found. Both sets also emphasized the probability that the psychosocial concomitants of TPP, especially the reactions of families and peers, contribute to the behavioral outcome.

Both of these articles prompt the editor to recommend that psychologists, psychiatrists, or others with special expertise in TPP closely monitor and counsel patients with sexual precocity.

Plasma GH and Sm-C Response to Continuous GHRF Infusion in Patients With GH Deficiency

The secretion of human growth hormone (hGH) is controlled by two hypothalamic hormones: growth-hormone–releasing factor (GHRF) and somatostatin. The release of these hormones is in turn controlled by neurotransmitters in the central nervous system. The role played by the two hormones and the neurotransmitters in the pulsatile secretion of hGH is not yet clear. It is only known that most of the growth hormone (GH) pulses occur during the first hours of deep sleep. With the investigations presented here, Hizuka et al aim at a better understanding of the regulatory mechanisms involved and an improved standard technique for the GHRF test.

In agreement with other investigators, the authors have shown previously that the majority of patients with idiopathic GH deficiency exhibit plasma GH increases following single or repetitive administration of GHRF-44. In the present protocol, the procedure is modified in favor of a combination of a ten-hour infusion of GHRF-44 at night with a subsequent bolus injection of the same hormone. Six patients with proven idiopathic GH deficiency underwent this protocol, receiving 0.5 μg/kg/h GHRF-44 during the infusion and, subsequently, 2 μg/kg as an intravenous bolus. Four other patients served as controls. They received a saline infusion over ten hours, followed by the same bolus injection.

In two of the patients receiving saline, no GH level increases were seen during saline infusion; in the other two, only minor increments were observed. Long-term GHRF infusion in the six patients significantly increased GH secretion. Four to 13 pulses were detected during sleep while the GHRF was being infused. The highest peaks varied from 3.5 to 10.3 ng/ml and the integrated GH secretions ranged from 13.1 to 40.2 ng/ml/h with a mean of 22.5 ng/ml. The subsequent bolus injections of GHRF induced GH increases in all ten patients. The peak levels observed in the patients after saline varied between 1.0 and 5.6 ng/ml, while levels after GHRF infusion varied between 2.5 and 13.5 ng/ml. The somatomedin-C values determined before and after GHRF were similar.

Hizuka N, Takano K, Shizume K, et al: *Acta Endocrinol* 1985;110:17-23.

Editor's comment—*The authors have shown that GHRF infusion at a dose of 0.5 μg/kg/h produced pulsatile GH secretion in patients with GH deficiency, and that the integrated area under the GH curve was much greater than that during saline infusion. With regard to the frequency of peaks, the secretion patterns resembled those previously observed in healthy subjects. However, with respect to the quantitative output, the secretion was much less, corresponding to approximately 30% of that seen in normal adults.*

Three aspects of these studies require comment. First, the hypophysis displays a pulsatile form of GH output, although the stimulating agent, GHRF, is administered continuously. This leads to the conclusion that the mode of pituitary GH secretion is pulsatile per se. *Second, the continuous administration of small amounts of GHRF for ten hours does not blunt the response to subsequent injections of standard doses of GHRF, whereas the infusion of larger amounts blunts the subsequent response (Vance et al:* JCEM *in press), which is probably due to refractoriness of the pituitary somatotropins. Third, somatostatin may be the controlling factor in GH secretion, since GHRF was infused at a constant rate in these studies of Hizuka et al, yet GH was released in a pulsatile fashion.*

Somatomedin-C and Thymidine Activity in Appropriate and Small-for-Gestational-Age Human Newborns

During the past ten years, several reports on serum growth factors in newborns and premature infants have been published. In this report, two well-defined groups of term newborns are compared, using two different procedures. Ten infants had appropriate birth weights for gestational age (AGA); $\bar{X}$ = 3,492 ± SEM 1,388. Eleven infants were small for gestational age (SGA) with a mean birth weight of 2,610 ± 46 g. Blood from the infants was obtained directly from neonatal vessels, rather than from the umbilical cord. Thymidine activity (TA) was determined by measuring the effect of the serum on thymidine incorporation into human lymphocytes activated by phytohemagglutinin. Somatomedin-C (Sm-C) was measured by radioimmunoassay (RIA) after separation from carrier protein. In addition, transferrin was determined using Mancini's technique of radial immunodiffusion.

The mean ± SEM results obtained in the two groups are shown in the table.

Measurements	AGA	SGA	*P*
TA (U/ml)	1.51 ± 0.08	1.04 ± 0.11	<0.001
Sm-C (U/ml)	0.52 ± 0.03	0.32 ± 0.03	<0.001
Transferrin (g/l)	1.69 ± 0.15	1.61 ± 0.13	Not significant

The TA values in the SGA newborns correspond to normal adult values (1.0 U/ml), whereas those of the AGA infants are 50% higher (1.5 U/ml). The Sm-C levels, by contrast, are markedly lower (0.52 and 0.32 U/ml) in both groups of infants than in normal adults (1.0 U/ml). The transferrin levels were similar in both groups and significantly below the mean adult level.

Thiériot-Prévost G, Doffos F, Forrestier F: *Acta Endocrinol* 1985;110: 32-35.

Editor's comment—*The results presented here agree with previously reported results, obtained by radioimmunologic as well as biologic methods. The advantage of this study, however, is its simultaneous application of both assays, thus permitting their immediate comparison. TA values were positively correlated with the Sm-C levels in the AGA newborns (r=0.72, P<0.05) but not in the SGA group. The significant difference of the TA values v the SM-C–RIA values suggests that Sm-C plays a major role in the growth factors determined as thymidine activity, but is certainly not the only substance generating growth-promoting activity, as reflected by thymidine uptake. The importance of other factors, including the embryonic somatomedin described by Sara et al (1981), remains to be elucidated.*

Obviously, there exists a relationship between impaired fetal growth and diminished Sm production and thymidine activity. Nevertheless, no individual correlation between the Sm levels and the birth weight was observed. The data on transferrin confirm previous investigations and demonstrate again that transferrin apparently does not play a direct role in fetal growth.

The entire subject of fetal growth and fetal growth factors remains a challenging field for investigation. Our understanding of the phenomena involved remains exceedingly limited.

Effects of Intravenous, Subcutaneous, and Intranasal Administration of GH–Releasing Hormone-40 on Serum GH Concentrations in Normal Men

The effects of intravenous (IV), subcutaneous (SC), and intranasal growth-hormone–releasing hormone 40 (GHRH-40) on growth-hormone (GH) secretion were measured in normal adult volunteers. To better define the dose-response relationship between GHRH-40 and secreted GH, the circulating levels of immunoactive GHRH-40 were quantitated. Normal men received either vehicle solution or GHRH-40 IV (0.003 to 0.1 μg/kg), SC (1 to 10 μg/kg), or intranasally (3 to 100 μg/kg). The table gives the results obtained during the two-hour period after IV administration or the three-hour period after SC or intranasal administration of GHRH-40.

In addition, significant dose-response relationships were documented between the maximal increments above basal in serum GH and GHRH-40 administered by all routes.

The mean peak plasma level of GHRH achieved after IV administration of 10 μg/kg GHRH-40 was approximately 60 and 500 times greater than the mean levels achieved after the same dose SC and intranasally, respectively.

Route of administration	Dose (μg/kg)	Maximal GH increment over basal (ng/ml)
Intravenous	0.1	15.5
Subcutaneous	3.3	26.2
	10	63.6
Intranasal	30	18.5
	100	21.7

Evans WS, Vance ML, Kaiser DL, et al: *JCEM* 1984;61:846-850.

Editor's comment—*If chronic GHRH therapy is to become a reasonable alternative to GH therapy, one must be able to give appropriate quantities by SC or intranasal routes. The present preparation is active SC when given as 1 to 3 μg/kg SC every three hours by micropump (see Thorner et al,* N Engl J Med *1985;312:4). The present data indicate that the intranasal route is not yet practical. If the intranasal route is to be used, what is clearly needed are more lipid-soluble analogs (a peptide composed of the first 29 amino acids of GHRH is biologically active) or the fabrication of a lipophilic delivery system that allows the peptide to cross biological membranes. The dose-response relationships confirm that levels of GHRH of 40 to 60 pg/ml are necessary to evoke GH secretion.*

MEETING CALENDAR

April 12-17 American Academy of Pediatrics. Spring Session. Orlando, Florida. Contact: American Academy of Pediatrics, Division of Continuing Education, P.O. Box 927, Elk Grove Village, IL 60067 (312-228-5005 or 800-433-9016)

April 28-30 1st International Symposium on Serum Hormone-Binding Proteins. Contact: Dr. M.T. Forest, INSERM U34, Hôpital Debrousse, F-69322, Lyon, Cedex 05, France

May 1-4 Postgraduate Course: Current Review of Pediatric Endocrinology. Washington, D.C. Contact: Dr. Salvatore Raiti, Suite 501-9, 210 West Fayette Street, Baltimore, MD 21201 (301-837-2552)

May 6-9 American Pediatric Society/ Society for Pediatric Research. The Sheraton Washington Hotel. Washington, D.C. Contact: William Berman, Jr., Department of Pediatrics, University of New Mexico School of Medicine, Albuquerque, NM 87131 (505-277-4361)

May 9 Annual Meeting of the Lawson Wilkins Pediatric Endocrine Society. The Sheraton Washington Hotel, Washington D.C. Contact: Dr. Salvatore Raiti, Secretary, LWPES, Suite 501-9, 210 West Fayette Street, Baltimore, MD 21201 (301-837-2552)

June 8-11 March of Dimes Birth Defects Foundation Clinical Genetics Conference (With Focus on Muscle), Symposium, Westin Bellevue-Stratford Hotel, Philadelphia, Pennsylvania. Contact: Dr. Roy D. Schmickel, Conference Chairman, University of Pennsylvania. c/o March of Dimes Birth Defects Foundation, 1229 Chestnut Street, Philadelphia, PA 19107

June 22-24 46th Annual Scientific Sessions of the American Diabetes Association. Anaheim Convention Center, Anaheim, California. Contact: American Diabetes Association, 2 Park Avenue, New York, NY 10016 (212-683-7444)

June 25-27 65th Annual Meeting of The Endocrine Society. Anaheim Convention Center, Anaheim, California. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

July 6-10 26th Meeting of the Teratology Society. Park Plaza Hotel and Towers, Boston, Massachusetts. Contact: Alexandra Ventura, Administrative Assistant, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-564-1493)

July 7-12 XVIII International Congress of Pediatrics. Sheraton Waikiki, Honolulu, Hawaii. Contact: Dr. Gerald E. Hughes, Director, Office of the International Congress of Pediatrics, American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

September 22-26 7th International Congress on Human Genetics. International Congress Center, West Berlin, Germany. Contact: Congress Bureau, DER-CONGRESS, Congress Organization, Augsburger Strasse 27, D-1000 Berlin 30 (Telephone: 030-24-60-11)

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Primary Hypophosphatemic Rickets And Growth Retardation

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Primary (hereditary) hypophosphatemia is the most common cause of rickets in the United States now that vitamin D deficiency is uncommon. Primary hypophosphatemia is also a cause of growth retardation, which may occasionally present before the deformities of rickets appear.

Winters and co-workers studied the genetics of primary hypophosphatemia in a large kindred. They concluded that the disorder was transmitted by a single gene as an X-linked dominant trait. In this kindred there was no male-to-male transmission, but all female offspring of affected males were also affected. Consistent with Lyon's hypothesis, the manifestations of the disorder were often less severe in females than in males.

Although the disorder in humans is often referred to as X-linked hypophosphatemic rickets, it has been transmitted by an autosomal dominant mechanism in other kindreds. In one such kindred studied by us, the affected father had one affected son, two affected daughters, and one unaffected daughter. No phenotypic differences could be detected between these individuals and those in kindreds in which an X-linked dominant gene was involved. Interestingly, a significant number of patients presenting with hypophosphatemic rickets have no family history of the disorder, which suggests a spontaneous mutation. Follow-up of several such individuals who have since had affected offspring has confirmed the new mutation theory.

Phosphate Concentrations and Renal Functions

The concentration of inorganic phosphate in plasma is determined primarily by renal function. The usual diet for humans is rich in

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Fetal Growth and Development: A Brief Survey of Cellular Mechanisms

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The growth and development of the human embryo and fetus result from an orderly series of events that occur between fertilization and birth and lead to the formation of many complex structures and a myriad of interrelating specialized functions.

Although there are many reports in the literature associating specific events and observations with modulation or alteration of fetal growth, much remains to be learned about the cellular and subcellular mechanisms governing fetal growth and development. However, recent advances in understanding the control of fetal growth have resulted from research designed to answer these fundamental questions:

- What substances stimulate cellular replication and/or differentiation?
- Do these agents act on all cells, or are specific factors required for each cell type?
- What governs the synthesis of growth regulators?
- How do these substances act on their target cells?

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In This Issue

Primary Hypophosphatemic Rickets and Growth Retardation

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phosphate, a component of both animal and vegetable cells that is found in high concentration in cow's milk. Intestinal absorption of phosphate is influenced by vitamin D. However, even in vitamin D-deficient states, sufficient phosphate is absorbed to provide for tissue and bone phosphate needs if the diet is high in phosphate and if renal excretion of phosphate is appropriately reduced. Hypophosphatemia due to failure of intestinal absorption of phosphate can be induced by a very high intake of an aluminum or calcium salt, which precipitates phosphate in the intestinal lumen. The only other situation in which hypophosphatemia develops despite normal renal function is that of the prematurely born infant who is fed human milk and whose phosphate intake is low.

The metabolic abnormality in primary hypophosphatemia is impairment of tubular reabsorption of phosphate in the proximal convoluted tubule, the major site of phosphate reabsorption. This sodium-dependent phosphate transport system has a maximal rate that varies somewhat with the rate of glomerular filtration. The phosphate Tm expressed as mg P absorbed per dL of glomerular filtrate is highly correlated with serum P expressed as mg/dL. Parathyroid hormone (PTH) inhibits tubular reabsorption of phosphate and is an important factor in *controlling* serum phosphate concentration in normal individuals.

Calcium and Vitamin D Concentrations

A defect of intestinal transport of phosphate is also present in primary hypophosphatemia and may be the basis of impaired absorption of calcium. Serum calcium concentrations are normal and PTH function, as measured by serum immunoreactive PTH concentrations (IPTH), is also normal. The only sign of impaired calcium absorption is low excretion of calcium in the urine. The eucalcemic state is due to the hypophosphatemia, which reduces the flow of calcium from extracellular fluid into bone. Indeed, the lack of phosphate prevents bone mineralization and results in rickets.

The osteomalacia seen in patients with hypophosphatemic rickets is not prevented by physiologic amounts of vitamin D. Measurement of the concentrations of vitamin D metabolites in the serum of such patients has shown normal concentrations of 25-hydroxyvitamin D as well as 1,25-dihydroxyvitamin D. However, the concentrations of the latter are usually at the lower limit of normal. Since reduction of extracellular phosphate has been found to activate 25-hydroxyvitamin D,1-hydroxylase in kidney tubule cells, the relatively low concentration of 1,25-dihydroxyvitamin D in patients with primary hypophosphatemia suggests that there is, in addition to the impairment of tubular transport of phosphate, a defect in the linkage between phosphate concentration and the formation of 1,25-dihydroxyvitamin D by tubule cells.

Clinical Features and Diagnosis

The **growth retardation characteristic of the untreated subject** is believed to be secondary to hypophosphatemia, although the specific mechanism by which reduced concentrations of inorganic phosphate in extracellular fluid interfere with cellular growth is undetermined. Only part of the **reduced height** of these patients can be ascribed to the **bending and twisting deformity of the lower extremities** resulting from the impaired mineralization of the metaphyseal cartilage and the growing bone. Since 9 mg of phosphorus is retained in the cell for each gram of protein synthesized, it is likely that phosphate deficiency can inhibit cell growth through reduction of intracellular organic phosphate. Further evidence of the relationship between extracellular phosphate concentration and linear growth is seen in the acceleration of growth following treatment, as described later in this article.

Reduced serum phosphate concentration is, of course, the basis of the diagnosis of primary hypophosphatemia. Since physiologic serum phosphate concentrations are much higher in infants and children than in adults, appropriate standards must be used. Serum phosphate concentrations below 4.5 P/dL in infants less than 3 or 4 months of age, as well as concentrations below 4 mg P/dL in older infants and children, should be regarded as abnormal.

If there is a **positive family history** for primary hypophosphatemia, **the diagnosis is confirmed by serial determinations of serum phosphate in the infant.** Prenatal diagnosis is not possible. Even if the mother is hypophosphatemic, placental transport of phosphate into the fetal plasma is not impaired. Thus, fetal growth and mineralization of the fetal skeleton are normal. For this reason, the diagnosis cannot be made in the early neonatal period either.

During the first weeks of life, the glomerular filtration rate is physiologically low and the serum phosphate concentration may remain in the normal range despite the phosphate reabsorption abnormality. Thus, unequivocal hypophosphatemia may not be detected until the infant is several months of age, particularly if the infant is fed a high-phosphate, cow's-milk-based formula.

Before a degree of hypophosphatemia that is considered to be diagnostic of the condition is reached, **serum alkaline phosphatase concentrations are often elevated.** This might be the initial evidence that the infant is affected and it presumably represents increased proliferation of osteoblasts in response to defective mineralization of bone. Possibly, the integrated 24-hour concentration of extracellular phosphate in these infants is below the concentrations necessary for mineralization of the rapidly growing skeleton even though a randomly determined serum phosphate level is not sufficiently low to be diagnostic.

If there is **no family history** of primary hypophosphatemia, the diagnosis is not usually made until **rachitic deformities** are noted. Since the most obvious deformities are in the lower extremities and develop after weightbearing, suspicion is not aroused until the second year of life **when bowing of the legs** (genu varum) or **knock-knee deformity** (genu valgum) develops, with the former being more common. Decreased serum phosphate concentrations, increased serum alkaline phosphatase levels, and

characteristic x-ray findings of irregular mineralization at the metaphyseal ends of the long bones rule out other causes of leg deformities such as Blount's disease, physiologic bowing, and chondrometaphyseal dysplasia. A history of adequate vitamin D intake and/or exposure to sunshine argues against vitamin D deficiency as a cause of the deformities.

In rare instances, calcium deficiency may account for the rickets seen in infants who cannot tolerate milk and have not been given calcium supplements when placed on a milk-free diet. Measurements of the vitamin D metabolites, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in serum, and the IPTH concentration will differentiate primary hypophosphatemia from calcium deficiency or from vitamin D-dependent rickets (ie, rickets caused by defective conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D or by lack of end-organ receptors for 1,25-dihydroxyvitamin D). As mentioned previously, serum IPTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D concentrations are in the normal range, as is the serum calcium level, in patients with primary hypophosphatemia.

Treatment

The treatment of primary hypophosphatemia consists of increasing phosphate intake to raise extracellular phosphate concentrations as well as taking adequate amounts of the active vitamin D metabolite, 1,25-dihydroxyvitamin D, or an analogous compound, dihydrotachysterol. The former is available commercially (as Rocaltrol) in capsules containing 0.25 or 0.5 μg of active metabolite. This formulation is inconvenient for infants, who can be more readily treated with a solution of dihydrotachysterol in oil.

Both compounds act to increase intestinal absorption of phosphate and calcium. Unless adequate amounts of these compounds are given, ingestion of large amounts of phosphate will sequester calcium, thus preventing its absorption, and result in secondary hyperparathyroidism as measured by increased serum IPTH concentration. The appropriate dose of 1,25-dihydroxyvitamin D or dihydrotachysterol is that amount necessary to bring calcium absorption into the normal range. Calcium absorption can be indirectly assessed by measuring urine calcium excretion and serum calcium concentration. Depressed intestinal absorption of calcium is indicated by low urine output of calcium, whereas hyperabsorption of calcium results in excessive excretion of calcium and increased serum calcium concentration. The normal range of calcium in the urine in children is 1 to 4 mg/kg/24 h. If 24-hour collections are not possible, the calcium/creatinine ratio of a single voided specimen can be used as an approximation, the normal range being 0.05 to 0.25 mg calcium per mg creatinine.

The dose of phosphate is 1 to 1.5 g of phosphorus in four or five divided doses for infants under 2 years of age and 2 or 2.5 g of phosphorus per day for older children. The preparations available are Neutra-Phos, Neutra-Phos-K, or K-Phos. My preference is for Neutra-Phos-K, which is buffered potassium phosphate.

Application of this treatment is often quite difficult. The large load of phosphate salt is unpalatable and its osmotic effect may cause hyperperistalsis and diarrhea. Some patients cannot tolerate the phosphate except in minimal dosage, but attempts must be made to increase the phosphate gradually until an adequate dose is achieved or tolerance is exceeded. Phosphate must be given in three or four divided doses per day to keep serum phosphorus levels in the normal range for a significant portion of each 24-hour period.

If treatment is adhered to, radiologically normal bone structure and excellent growth can be attained, as shown in Figure 1. L.W., the daughter of a hypophosphatemic mother who was 59 inches in height, was

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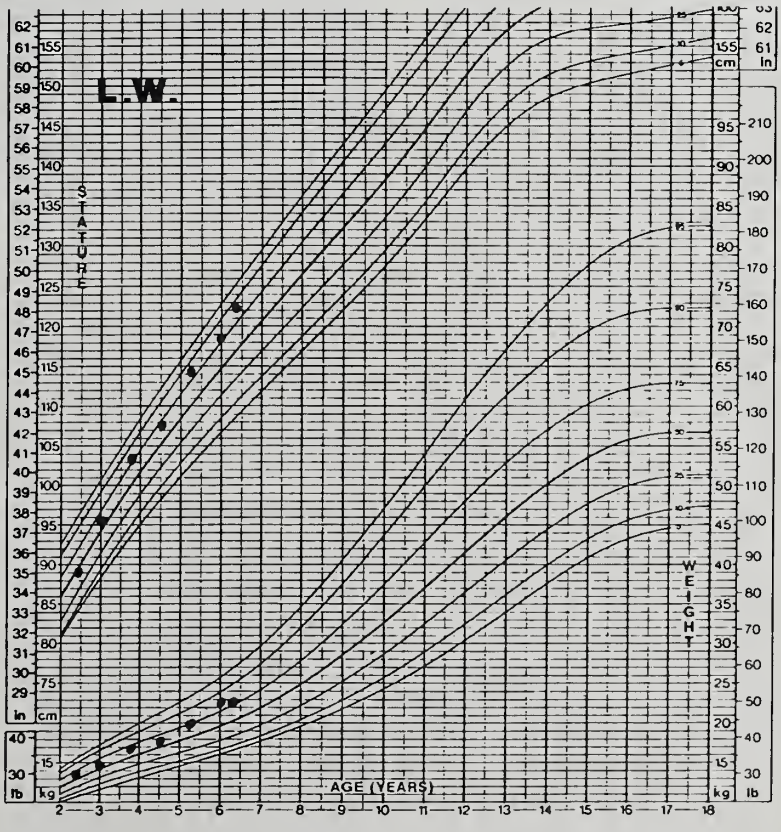
Figure 1. Growth curves of a child who was diagnosed as having primary phosphatemia at two months of age. Treatment was begun at the time of diagnosis.



Adapted from Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth. National Center for Health Statistics percentiles. *Am J Clin Nutr* 32:607–629, 1979 Data.

Primary Hypophosphatemic Rickets and Growth Retardation

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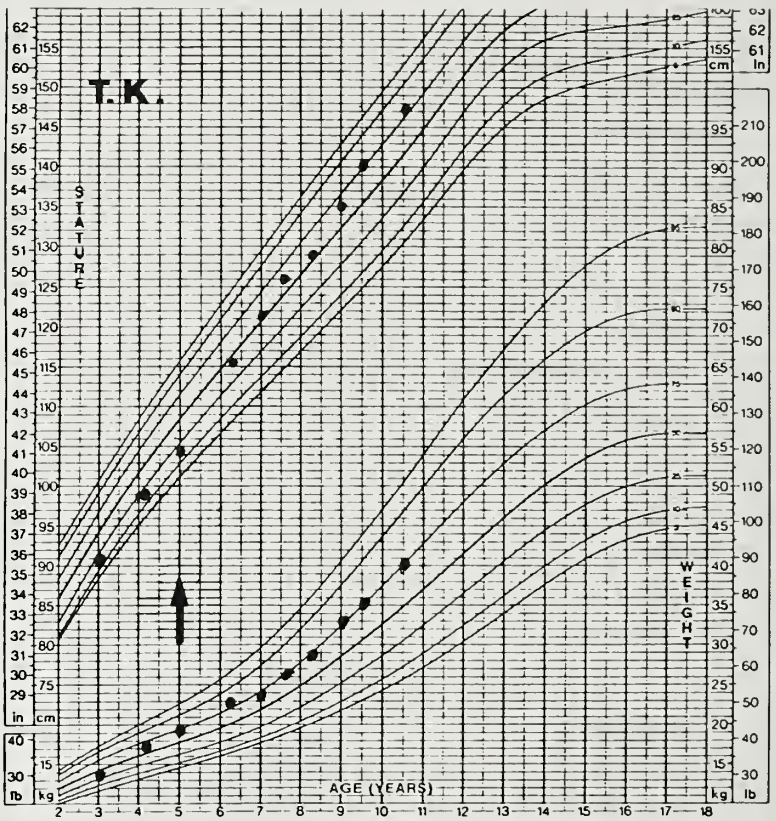
diagnosed as having primary hypophosphatemia at 2 months of age. Treatment was begun as indicated above. The child tolerated treatment, and there was excellent compliance.

In the case of T.K. (Figure 2), the family history was negative for hypophosphatemia. At 2 years of age, T.K. had marked genu varum and x-ray evidence of rickets, but a specific diagnosis was not made and appropriate treatment was not begun until she reached 5 years of age (indicated by the arrow). These two patients are admittedly the exceptions rather than the rule, but they clearly illustrate how proper treatment can improve growth and bone mineralization.

Figure 2. Growth curves of a child who did not begin receiving treatment for primary phosphatemia until she was 5 years of age (the arrow denotes the beginning of therapy). Although this child exhibited clinical signs and radiologic evidence of rickets at the age of 2 years, a definitive diagnosis was not made until three years later.



Adapted from Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth. National Center for Health Statistics percentiles. *Am J Clin Nutr* 32:607–629, 1979 Data.

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Fetal Growth and Development: A Brief Survey of Cellular Mechanisms

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The term "growth factor" has been used as a generic designation for any substance capable of inducing cellular proliferation and/or differentiation. Some growth factors act on a wide variety of cell types, while specific cells are the targets of others. All of the known growth factors may be important to the fetus. This survey will review some of the known growth factors and their actions on fetal growth, as well as point out their regulation by classical hormones and their probable paracrine and/or autocrine mechanisms of action. Insights into growth mechanisms derived from the study of oncogenes will also be explored, and speculation regarding the course of future research in fetal growth will be offered.

Growth Factors With Broad Specificity

Epidermal growth factor (EGF), a 53-amino-acid peptide measuring 6,045 daltons (da), is a potent mitogen for cells of ectodermal and mesodermal origin. In the fetal lung, EGF stimulates branching morphogenesis and the proliferation of airway epithelium. It also appears to be involved in skin growth. EGF can be measured in amniotic fluid and is present in high concentrations in human milk, the latter suggesting that it might have a role in gut maturation. Tissue concentrations of EGF are regulated by androgens and thyroxine.

The somatomedins, or insulin-like growth factors, somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) and IGF-II, are peptides of 7,649 and 7,471 da, respectively. They are potent mitogens for a wide variety of cell types and have marked amino acid sequence homology with insulin. Both appear to be synthesized in many fetal tissues, and cell surface receptors that presumably mediate their mitogenic actions are ubiquitous in the fetus. The fetal rat has high blood concentrations of multiplication stimulating activity (MSA, the rat homologue of IGF-II) and abundant MSA cell surface receptors, suggesting an important role in prepartum rodents. While it is clear that Sm-C/IGF-I and, to some extent, IGF-II as well, are regulated postnatally by growth hormone (GH) and nutritional status, placental lactogen may supplant GH in the fetus. It is also likely that nutrient supply plays an important role.

Platelet-derived growth factor (PDGF), a disulfide-linked, two-chain protein of approximately 30,000 da, is one of the several mitogens found in platelets. PDGF stimulates cellular proliferation in concert with other peptide mitogens, such as EGF and Sm-C/IGF-I. Based on experiments in cultured cells, it is now thought that PDGF is one member of a family of peptides that act by making resting cells capable of undergoing DNA synthesis when they are stimulated by other mitogens.

Fibroblast growth factors and endothelial growth factors are examples of mitogens with PDGF-like effects. The former has been purified from bovine brain and acts on cells of endodermal and mesodermal origin, while the latter are proteins purified from brain and platelets that presumably act specifically on vascular endothelial cells. PDGF is probably important in wound healing, given its high concentrations in platelets and their known role in clotting. In the fetus, peptides with PDGF-like actions may be necessary to stimulate the proliferation of many cell types.

Growth Factors for Specific Cells

Erythropoietin, an acidic sialoprotein containing 166 amino acids, appears to be made in the kidney. It stimulates the mitosis and differentiation of red cell precursors in response to hypoxia. Its production is also stimulated by androgens and GH. Colony-stimulating factors are peptides and glycoproteins derived from a variety of tissues that stimulate white blood cell proliferation and differentiation.

Hematopoietic cells also synthesize several mitogens, of which the interleukins are perhaps the best described. Interleukin-1 is the designation for a group of peptides made by macrophages and other cells that are capable of a variety of actions on both T and B lymphocytes. Interleukin-2, a 15,500-da peptide, is made by T lymphocytes and promotes their proliferation after antigenic stimulation. Interleukin-3, a 28,000-da glycosylated protein, stimulates the growth of immature lymphocytes.

Thymosins and thymopoietins are peptides made in the thymus; they promote the growth and differentiation of immunologically competent lymphocytes. Macrophage growth factors have also been described. They are made by mononuclear phagocytes and appear to exert their mitogenic effects on a variety of other cell types.

Growth Factors Causing Differentiation

Nerve growth factor (NGF) is the best characterized of the growth factors that promote cellular differentiation. The biologically active form, β NGF, is a 13,259-da peptide that is 25% homologous with insulin. NGF promotes the survival, differentiation, and axonal outgrowth of sensory and sympathetic ganglia. It appears to be synthesized in the peripheral tissues that are innervated by these ganglia and is transported by retrograde movement to cell bodies where the signals for axonal growth are given.

Thyroxine may be essential for NGF synthesis in the fetal and neonatal central nervous system. When thyroxine blood concentrations are low in the neonatal rat, brain NGF concentrations are reduced. This may explain why hypothyroid fetuses and newborns have impaired brain development and neurologic handicaps.

Fibroblast pneumonocyte factor (FPF) is another example of a substance capable of differentiative action. It is made by lung fibroblasts in response to glucocorticoids and stimulates surfactant production by type II pneumonocytes.

Other Stimulators of Fetal Growth

The study of cellular oncogenes and the proteins they encode provides clues to normal growth mechanisms in the fetus. Cellular oncogenes are stretches of genomic DNA (genes) containing nucleotide sequences that are homologous with nucleotide sequences of RNA in retroviruses

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isolated from naturally occurring tumors. These viruses, whose genetic information is encoded by RNA, can transform normal cells into neoplastic cells. Retroviruses possess enzymes, called reverse transcriptases, that are capable of transcribing RNA into DNA. Therefore, the genetic information encoded in the RNA of these enzymes can be incorporated into the DNA of the infected host cell. The portion of the retroviral RNA responsible for neoplastic transformation is termed the viral (v) oncogene.

It is now thought that v oncogenes originated from normal cellular genes, presumably by recombinational events occurring during the viral transfection of normal cellular DNA. By virtue of their presence in whole or in part in the genome of the retrovirus, these normal genes are called cellular proto-oncogenes, despite the fact that they are normal genomic components and, as such, have no role in oncogenesis. The proteins that these genes encode are called proto-oncogene proteins (pOGP). Because proteins encoded by v oncogenes are responsible for the abnormal growth of neoplastic cells, it follows that the pOGPs may be important in normal cellular proliferation. For example, the v oncogene *v-sis* is part of the RNA genome of the simian sarcoma virus and encodes a protein designated *p28-sis*. This protein is almost identical to the 109 N-terminal amino acids of the β chain of PDGF. The normal cellular proto-oncogene that encodes this protein is called *c-sis*.

There are many other examples of proteins encoded by v oncogenes that have normal cellular counterparts. Identification of v oncogenes and the proteins they encode is, therefore, likely to lead to a better understanding of normal growth mechanisms by revealing other proteins and their genes that are important in this process.

Other links between normal cellular growth and tumor growth come from the study of substances termed transforming growth factors (TGF). TGF_{α} is a 51-amino-acid peptide that was purified from the media of normal rat kidney cells after they were transformed by the murine sarcoma virus. TGF_{α} shares 44% homology with human EGF, and when added to normal cultured cells together with another substance called TGF_{β}, it induces transformation. TGF_{α} has been identified in many normal cells, suggesting that it may be a normal EGF-like growth factor.

Difficulties in the Study of Fetal Growth

Until recently, many studies of the regulation of fetal growth focused on classical hormones. Endocrine mechanisms of regulation were anticipated. Specifically, it was expected that substances synthesized at sites distant from their sites of action would control growth. Few growth factors, however, appear to act in this manner. Rather, they act in a paracrine or autocrine fashion. Their sites of synthesis are distributed widely and their biologic effects occur on nearby cells or on their cells of origin.

While classical hormones are synthesized by and have biologic actions in the fetus, clear regulatory roles, such as those defined in postnatal growth, have been difficult to define in the fetus. Aside from the inaccessibility of the fetus, at least two reasons for this difficulty are apparent. Because the placenta can synthesize homologues of many classical hormones, it is difficult to distinguish *experimentally* the effects of hormones derived from the fetus and those derived from the placenta (and possibly from the mother).

In addition, many or most of the growth-promoting effects of classical hormones seem to be mediated by growth factors that act on the same tissue that produced them. For example, the maturational effect of thyroxine on the skin and central nervous system seem to be mediated, at least in part, by EGF and NGF, respectively. Growth stimulation by GH and placental lactogen is thought to be mediated by the somatomedins. Because growth factor synthesis and action in the fetus are likely to be dependent on multiple influences, the regulatory effects of classical hormones on growth factors are neither obvious nor easily delineated.

Mediation of the Action of Growth Factors

For peptide growth factors to stimulate developmental change, the cellular apparatus effecting the biologic response must also be in place. All peptide growth factors are thought to initiate their actions by binding to cell surface receptors. In most instances, this is followed by phosphorylation of the receptor, as well as certain cytosolic proteins. Subsequently, a series of biochemical events occurs, leading either to mitosis or differentiation of the target cell. The precise nature of the events occurring in response to receptor binding has not been delineated completely. Evidence that such events are crucial is provided by findings that many proteins encoded by oncogenes and cellular proto-oncogenes are kinases, enzymes that phosphorylate proteins. Similarly, at least one v oncogene product (gp65 encoded by v *erbB*) is homologous to a portion of the EGF receptor.

The Future of Research on Fetal Growth and Development

To understand fetal growth, we must understand the mechanisms that control transcription and translation of each protein involved in the developmental process. Important questions to be answered include:

- What mechanisms govern transcription of the genes that encode proteins that are important in development?
- How is transcription coordinated with the development?
- What turns the genes that are important in development on and off?
- What translational and post-translational events influence the gene product that is expressed at each phase of development?

In addition, we must understand the regulation of structure. Important information about the genes regulating the gross morphology of one complex species, *Drosophila melanogaster,* is now accruing. Within a 70-kilobase DNA sequence, termed the bithorax complex, there are specific genes that govern the development of the three thoracic and eight abdominal segments of the fruit fly. By investigating the genome of *Drosophila* mutants

that lack somatic segments or have segmental abnormalities, the DNA sequences (genes) that regulate some specific segments have been deduced. Messenger RNAs encoded by these genes are now being isolated and it is hoped that the expressed proteins will be identified. This will allow further study of mechanisms that lead to gross structural morphology. Perhaps the elucidation of mechanisms governing the sculpturing of the fruit fly will provide a basis for similar studies in man.

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The Effect of Adrenal Androgens on Skeletal Maturation and Growth

There are few instances when the effect of gonadal steroids on growth and skeletal maturation can be distinguished from those of adrenal steroids. Wierman et al have elegantly examined the interrelationship of adrenal and gonadal function in 29 patients with sexual precocity by measuring DHAS, a steroid produced almost exclusively in the adrenals, in these patients before and during treatment with the LHRH analogue (LHRHa [D-Trp 6-Pro 9-NET]). The authors have correlated the findings with changes in skeletal maturation and predicted height, and they have established the following salient points:

(1) Only 10 of 29 patients studied had coincident premature adrenarche as determined by adolescent values of DHAS (≥60 μg/dl).

(2) The use of LHRHa did not alter the DHAS levels in patients with adrenarche.

(3) The predicted heights of patients with sexual precocity *but without* adrenarche increased significantly more than those with adrenarche as a result of LHRHa therapy.

(4) The change in bone age/change in chronological age ratio was greater over a period of 1 to 4 years in patients with associated adrenarche than in those without.

(5) The presence of pubic hair did not correlate with DHAS levels before therapy.

(6) Sexual hair regressed in patients without adrenarche when treated with LHRHa but not in those with adrenarche.

On the basis of these findings the authors conclude:

(1) Adrenarche is not under the control of gonadotropins and the factor(s) that induce adrenarche remain obscure.

(2) The data presented suggest that adrenal androgens contribute significantly to epiphyseal advancement during adolescence.

(3) LHRHa therapy is potentially most effective in increasing the height of children with sexual precocity if they do not have adrenarche in association with the sexual precocity.

Wierman ME, Beardsworth DE, Crawford JD, et al: *J Clin Invest* 1986;77:121.

Editor's comment—*This paper is well worth reading and digesting completely. It presents data that provide insight into the separate occurrences of gonadarche and adrenarche and it also reviews what we currently know and do not know about the relationship of adrenarche to skeletal maturation.*

Fetal Alcohol Syndrome: Two Reports

I. Natural History: A Ten-Year Follow-up of 11 Patients

In 1973, Jones et al described 11 children with a common pattern of altered morphogenesis and central nervous system dysfunction. Their mothers were chronic alcoholics who continued to drink heavily during pregnancy. Since then, fetal alcohol syndrome has been identified in children from every racial group and in many countries. The teratogenicity of alcohol has been confirmed in laboratory studies involving many different species of animals, and a dose-response curve for prenatal alcohol exposure has been established.

In this report, the authors describe how the 11 children have developed physically and mentally over the past ten years. Two are now dead, one is lost to follow-up, and the remaining eight continue to be growth deficient (with respect to height, weight, and head circumference) and dysmorphic. Although most showed some catch-up linear growth during the first year of life, weight and head circumference decreased relative to the norms during this time in most of the children. Thereafter, length and head circumference remained relatively constant with respect to the norms, whereas there was some catch-up in weight with increasing age. There was relatively slow growth of the head after delivery. During childhood, the children were all strikingly underweight for height.

The major craniofacial features—especially the short palpebral fissures, hypoplastic philtrum, thin vermilion border of the upper lip, and flat midface—did not change during the ten years of follow-up. However, their noses changed, with more prominent growth of the nasal bridge. Cardiac anomalies, which consisted of an atrial septal defect in one patient, patent ductus arteriosus in another, and a grade 3/4 systolic murmur interpreted as a ventricular septal defect in six, have all resolved spontaneously or have become insignificant. Orthopedic complications were managed successfully in almost all patients by casting or splinting.

The short palpebral fissures are thought to be secondary to the decreased growth of the eye. Frank microphthalmia was observed at necropsy in one of the patients. Chronic serous otitis media, probably secondary to eustachian tube dysfunction associated with maxillary hypoplasia, required medical and surgical procedures in four of the children.

None of the eight children followed had normal intellectual development. Four were mildly and four were seriously retarded. The degree to which postnatal environmental factors influenced the development of these children is difficult to assess. Mothers of three of the four seriously retarded children were so severely alcoholic that they died of alcohol-related causes within six years of giving birth.

The two major predictive factors concerning prognosis were the severity of the maternal alcoholism and the extent and severity of the initial pattern of malformation. The four children with the most striking craniofacial abnormalities had the most severe degree of microcephaly, the shortest stature, and the lowest intellectual function. The severity of maternal alcoholism appeared to be the most predictive factor in the backgrounds of the four most severely retarded children.

Streissguth AP, Clarren SK, Jones KL: *Lancet* 1985;2:85-91.

II. Prospective Study of Children Exposed to Variable Amounts of Alcohol in Utero

Although it is well known that offspring of mothers who consume large quantities of alcohol during pregnancy are at high risk for physical and mental deficiencies, few prospective studies have dealt with the fetal effects of interrupted alcohol consumption during pregnancy as the result of an intervention program. The authors describe a Swedish antenatal program that was started to help pregnant women stop alcohol abuse with the hope of reducing the adverse effects of alcohol on the fetus.

A total of 40 children born to alcoholic women (Groups 2 and 3) and 40 children born to nonalcoholic women (Group 1) attending the same local maternity health clinics for antenatal care were studied between the ages of 18 and 27 months. The mothers in Group 1 drank less than 30 g of pure alcohol prior to the first prenatal visit and abstained or minimized their consumption thereafter. Group 2 consisted of 25 children born to women who were classified as excessive drinkers and had an average consumption of 30 to 150 g of pure alcohol per day during the month before their first visit to the clinic. All mothers in this group markedly reduced their alcoholic consumption after their first visit and 19 abstained completely. Group 3 consisted of 15 children of alcoholic mothers who had an average consumption of more than 125 g of pure alcohol per day during the month before the first visit to the clinic. Nine mothers in this group stopped drinking alcohol during the first or second trimester, but the remaining six continued drinking throughout the pregnancy.

A statistically significant reduction in weight, height, and head circumference was seen in Group 3 children when compared with Group 1 children. Six of the 15 alcoholic women (Group 3) continued to abuse alcohol throughout pregnancy. Three of these women gave birth to children with abnormalities characteristic of fetal alcohol exposure; one child had the complete fetal alcohol syndrome. Only one child in Group 3 was normally developed in all physiological parameters and had normal behavior.

No fetal growth retardation was found among the children in Group 2, where the mothers reduced or ceased alcohol consumption after their first prenatal visit. Neither did these children show any other physical or physiological characteristic of fetal alcohol syndrome. About half of them, however, had retarded speech that the authors attributed to postnatal environmental influences. Indeed, signs of social instability, such as frequent separations between the parents and frequent registrations with the social welfare department, were seen in Group 2.

The authors suggest that fetal exposure to alcohol has a severe adverse effect on development, but

this can be significantly reversed by abstaining from alcohol in the first trimester of pregnancy. Cessation of alcohol abuse after the first trimester cannot reduce the documented increased risk of congenital malformations.

Larsson G, Bohlin AB, Tunell R: *Arch Dis Child* 1985;60:316-321.

Editor's comment—*Fetal alcohol syndrome has been well established as an important cause of congenital malformations, mental retardation, and prenatal and postnatal growth retardation. This syndrome is being diagnosed more frequently now that physicians are specifically questioning mothers-to-be about teratogenetic exposure and, especially, alcohol exposure. The study by Streissguth et al indicates the relationship between the severity of the maternal alcoholism and the severity of the physical and mental handicap. It also points out that the various features of this syndrome tend to be correlated to severity: degree of mental retardation, growth deficiency, and intellectual impairment. This study also points out the difficulty in diagnosing this syndrome after mid-childhood, since some of the typical facial characteristics change, specifically, the structure of the nose, while the markedly underweight appearance of the female children who had reached puberty disappeared. It is unknown whether this applies to the weight of males of pubertal age, since none of the boys had reached puberty. Moreover, it becomes increasingly difficult to obtain a documented history of maternal alcohol abuse as the children grow older.*

The Swedish study documents the importance of discontinuing alcohol before pregnancy or at least during the first few weeks of pregnancy. It is interesting that those women who drank fairly heavily during the first month or so of pregnancy and then stopped, or markedly reduced their intake, had no physical abnormalities in their children. Those women who did not stop or reduce their drinking until the second or third trimester, however, continued to have children with abnormalities characteristic of fetal alcohol exposure. It is hoped that with the increased emphasis and warnings concerning alcohol exposure in utero, the incidence of this devastating syndrome will be reduced in coming years.

Reevaluation of Russell-Silver Syndrome

Russell-Silver syndrome is characterized by intrauterine growth retardation (IUGR) and postnatal growth retardation in association with asymmetry of the body, normal head size, triangular facies, and normal psychomotor development. In 1953, Silver first described two patients with IUGR, body asymmetry, and postnatal growth retardation. In 1984, Russell described another group of five patients who also had growth deficiency, triangular facies, and disproportionate shortening of the upper limbs. Only two of the five had limb asymmetry. Since these two patients appeared to have the same anomalies described by Silver, the clinical entity was named the "Russell-Silver syndrome."

Saal et al reevaluated 15 patients with Russell-Silver syndrome 2.9 to 13 years after their initial diagnosis. They observed great variability in each of the features of the syndrome, suggesting that Russell-Silver syndrome is not a discrete entity but a heterogeneous group of disorders. Most interesting are the data regarding eventual growth. Five of the 15 exhibited late catch-up growth and attained normal heights. Eight remained below the third percentile, but paralleled the growth curve. Six of the 15 had gross evidence of body asymmetry at the time of diagnosis—four of the six continued to have a discrepancy in leg and/or arm length of more than 1 cm. One of the four had severe scoliosis.

Psychomotor development was abnormal in six of the 15. One of the six had seizures and six had café au lait spots, strongly suggesting neurofibromatosis. Psychomotor development in Russell-Silver syndrome had previously been thought to be normal, despite the frequent finding of gross motor delay in infancy. Eventual head circumference was normal in some and below the second percentile in others, unrelated to the degree of psychomotor delay. Russell-Silver patients are generally thought to have a normal head circumference. Eleven of the 14 patients originally described as having triangular facies were again so described on follow-up. Although hypogonadism and abnormal genital development had been described in several patients with Russell-Silver syndrome, all of these 15 patients had normal sexual development.

The authors conclude that the features of Russell-Silver syndrome are so diverse that it is highly probable the entity is a heterogenous group of disorders. It is thus difficult to offer parents a clear prognosis. In addition, if the diagnosis is made too loosely, the work-up for short stature may be prematurely terminated in some children; potentially correctable conditions could therefore be overlooked.

Saal HM, Pagon RA, Pepin MG: *J Peds* 1985;107:733.

Editor's comment—*Although the Russell-Silver syndrome has been considered a well-defined form of IUGR, it is clear that patients with a variety of forms of prenatal growth retardation have been lumped under this term. Conflicting reports of responsiveness to growth hormone therapy probably reflect this heterogeneity, especially in view of the fact that one third of the patients in this series obtained normal height without therapy. In the absence of a specific laboratory diagnostic test, the delineation of heterogeneity within a syndrome is difficult, but must always be kept in mind when offering parents a prognosis. In addition, the authors are correct in emphasizing that Russell-Silver syndrome can occur concomitantly with hypopituitarism or other causes of growth retardation. Cassidy et al recently reported (*AJDC *1986;140: 155) the seventh case of growth hormone deficiency in association with Russell-Silver syndrome.*

Computed Tomography of the Foramen Magnum: Achondroplastic Values Compared to Normal Standards

Achondroplasia, the most common of the skeletal dysplasias, is an autosomal dominant disorder whose clinical manifestations include short-limbed dwarfism, large head, shallow thoracic cage, and characteristic radiographic findings. This disorder is characterized by a decreased rate of endochondral ossification and normal membranous ossification.

The foramen magnum has long been recognized to be small in achondroplastic individuals. The small size is secondary to deficient growth of the endochondral exoccipital, supraoccipital, and basioccipital bones, which form the boundaries of the foramen magnum. Neurologic abnormalities seen in achondroplastic patients have included respiratory embarrassment, quadripareis and paraparesis, obstructive hydrocephalus, and sudden death. Compression of the upper cervical spinal cord and caudal medulla due to the small foramen magnum has been seen at autopsy in several cases. Surgical decompression by suboccipital craniectomy and cervical laminectomy has been suggested for patients with evidence of neurological involvement.

In this report, the extent of foramen magnum stenosis in achondroplasia was quantified by measurement of the maximal transverse and saggittal lengths of the foramen magnum by computed tomography (CT) scan. These were compared against foramen magnum measurements for persons of normal stature.

CT scans of the foramen magnum were performed by scanning at 0° horizontally from the top of the hard palate through the foramen magnum to the occiput. Maximum transverse and saggittal foramen magnum length was measured on the axial bone window scan with a ruler. Measurements were obtained from people of *normal stature* and varying ages: the transverse dimension was measured in 164 cases and the saggittal dimension in 144. Mean normal values ±1 SD were calculated at monthly intervals from birth through 2 years of age and at two-year increments thereafter. It was found that the mean foramen magnum size did not change appreciably after 15 years of age.

These values were then compared with foramen magnum measurements from 63 patients with achondroplasia. Among this group, 41 patients had no neurological findings. Twenty-two had evidence of neurological dysfunction suggestive of foramen magnum compression based on history, physical exam, short latency somatosensory potentials, and/or polysomnography.

From the data, one can conclude that the normal foramen magnum grows rapidly in both dimensions from birth to 1 year of age and then continues at a greatly diminished rate until approximately 15 years of age. The foramen magnum in achondroplastic individuals was significantly smaller than that of normal people at all ages. Achondroplastics without neurological dysfunction had measurements within ±5 SD of the normal mean for the transverse and ±4 SD for the saggittal dimensions. Patients with neurological symptoms had significantly smaller measurements. Included in this latter group were seven patients with extremely small foramen magnum size and obstructive hydrocephalus. Significantly, foramen magnum size was not shown to correlate with head circumference in this or any other group studied.

The authors concluded that stenosis of the foramen magnum may be more widespread in achondroplasia than had been previously appreciated. Because significant morbidity and potential mortality are associated with this stenosis, CT scans may identify individuals at high risk for these complications. However, the efficacy and safety of surgical decompression has not yet been clearly documented. The authors recommend that CT scan of the foramen magnum be considered part of the comprehensive care of individuals with achondroplasia.

Hecht JT, Nelson FW, Butler IJ, et al: *Am J Med Genet* 1985;20:355.

Editor's comment—*The recent recognition of late infantile mortality as a significant complication of achondroplasia has made careful neurological surveillance of achondroplastic children imperative. This report documents the differences in foramen magnum dimensions between normal and achondroplastic children, and especially between achondroplastic children with and without neurological complications. Newer diagnostic imaging procedures, such as magnetic resonance imaging (MRI) scans, have also become useful in the evaluation of spinal cord compression due to upper cervical vertebrae or foramen magnum stenosis in achondroplasia or other skeletal dysplasias. Unlike CT scanning, MRI can easily distinguish among varying soft tissue densities and can identify kinking or impingement on the spinal cord at an early stage. Discovery of anatomic abnormalities should be followed up by neurological and neurophysiological evaluation, including somatosensory-evoked potentials. Definitive criteria for neurosurgical intervention, however, remain to be established.*

Impaired Calcitonin Secretion in Patients With Williams Syndrome

The Williams syndrome (WS) is characterized by prenatal and postnatal growth retardation, microcephaly, facial dysmorphism (the so-called elfin facies), congenital heart disease (most commonly, supravalvular aortic stenosis), and mental deficiency. In a number of patients, this condition has been associated with neonatal hypercalcemia. This latter finding led to the initial description in 1952 of WS as "idiopathic hypercalcemia of infancy." In older children, metastatic calcium deposits have been found in the kidney, and osteosclerosis has been seen on x-ray in the skull and the metaphyses of the long bones.

Because the etiology of the hypercalcemia seen in WS has been debated for a number of years, the authors examined several aspects of calcium and vitamin D metabolism in five children with WS and seven age-matched controls. At the

time of this study, all patients, whose ages ranged from 2 to 14 years, had normal serum calcium concentrations. After an overnight fast, all received a 3 mg/kg bolus of calcium (as calcium chloride) and blood samples were analyzed over a one-hour period for serum calcium, parathyroid hormone (PTH), and immunoreactive calcitonin. In addition, the response of WS patients to an infusion of 200 U of synthetic human PTH was determined by measuring 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels. Serum calcium and phosphate concentrations were also measured at three and 24 hours after PTH administration.

Patients with WS had a delayed clearance of infused calcium when compared to the controls. No significant difference between the groups was noted in the PTH response, but the WS patients had a blunted immunoreactive calcitonin response. Serum creatinine, phosphate, and total protein values did not differ between the two groups. Finally, the rise in 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations seen at three and 24 hours after the PTH infusion was not significantly different in patients with WS from the response reported in normal adults.

The authors conclude that delayed clearance of the exogenously infused calcium in WS patients is due to deficient secretion of calcitonin rather than abnormalities of PTH or vitamin D metabolism. In support of their conclusions, they point out that children with hypothyroidism secondary to thyroid dysgenesis have a similar delay in clearance of infused calcium and a blunted calcitonin response. These children had little or no functional thyroid tissue, and presumably lacked calcitonin-secreting parafollicular cells (C cells).

Culler FL, Jones KL, Deftos LJ: *J Peds* 1985;107:720.

Editor's comment—*This study provides a possible explanation for the hypercalcemia associated with Williams syndrome. The authors have shown that the abnormality in calcium metabolism seen in patients with WS and thyroid dysgenesis might both result from a common pathophysiologic mechanism, namely, a deficiency of calcitonin secretion. The pathogenesis of the calcitonin deficiency in WS and its relationship to its other clinical manifestations remain unknown. It is interesting that the calcitonin deficiency was present in these normocalcemic older children, whereas the hypercalcemia seldom manifests itself after infancy. To better understand the physiology, calcitonin secretion should be evaluated prospectively in infants with WS and concomitant hypercalcemia.*

Letter to the Editor

Celiac Disease and Short Stature

In response to comments in the June 1985 issue of *Growth, Genetics, and Hormones,* I think you will find the following of interest. I recently reported [in the *American Journal of Gastroenterology,* October 1985, Volume 80, Number 10] a patient with what I believe to be the first asymptomatic celiac disease with short stature reported in this country.

The patient, a 6-year-old boy who was referred to me because of short stature, had no gastrointestinal (GI) symptoms except for a history of mild diarrhea during the first year of life. He was also biopsied by my colleague Dr. Hagos Tekeste on three separate occasions.

Initial evaluation showed a growth velocity of 4 cm/yr, a normal growth hormone (GH) response to clonidine stimulation, a low serum somatomedin-C (Sm-C) level, normal D-xylose absorption, normal quantitative stool fat collection (on a diet containing 50 mg of fat), and a small bowel biopsy consistent with celiac disease.

Adherence to a gluten-free diet restored the biopsy to normal and improved the growth velocity to 6 cm/yr. The patient's Sm-C level, which had been at levels consistent with GH deficiency prior to treatment, rose promptly after gluten was excluded from his diet. Anti-gliaden antibodies were not obtained, but are presently being measured by another physician because the patient has moved to another area since the report was made.

I am convinced that this is a very common disorder and that many of the so-called variant short stature group described by Rudman and others—particularly those with low Sm-C levels—may, in fact, be patients in this category.

Gerald H. Holman, M.D.
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Dr. Blizzard's Comments

Dr. Holman's letter was prompted by two discussions of celiac disease that appeared in Volume 1, Number 2 of *Growth, Genetics, and Hormones.* The abstract of Cacciarri (*J Peds* 1983;103:708) related that short stature was caused by celiac disease in 8.3% of children in an asymptomatic group of 60 short children.

In his article on nutrition, growth, and growth failure, Dr. Lifshitz pointed out that much attention has been given in Europe to the association between celiac disease without significant GI symptoms and short stature. Lifshitz believes that it is reasonable to suspect celiac disease in children who are short without an adequate explanation. He recommends that suspect patients be challenged with a high-gluten diet for four to six weeks and that an intestinal biopsy to confirm the diagnosis of celiac disease be done as well.

Readers of *Growth, Genetics, and Hormones* are encouraged to write and share their clinical experiences and interesting cases with their colleagues.

MEETING CALENDAR

July 6-10 26th Meeting of the Teratology Society. Park Plaza Hotel and Towers, Boston, Massachusetts. Contact: Alexandra Ventura, Administrative Assistant, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-564-1493)

July 7-12 XVIII International Congress of Pediatrics. Sheraton Waikiki, Honolulu, Hawaii. Contact: Dr. Gerald E. Hughes, Director, Office of the International Congress of Pediatrics, Americar Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

September 12-13 8th Annual Emergency Pediatrics. Royal Sonesta Hotel, Cambridge, Massachusetts. Contact: Department of Continuing Education, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118 (617-638-4605)

September 22-26 7th International Congress on Human Genetics. International Congress Center, West Berlin, Germany. Contact: Congress Bureau, DER-CONGRESS, Congress Organization, Augsburger Strasse 27, D-1000 Berlin 30 (Telephone: 030-24-60-11)

October 16-18 Pediatric Nutrition Update. San Francisco, California. Contact: Extended Programs in Medical Education, University of California, Room U-569, San Francisco, CA 94143-0742 (415-476-4251)

November 1-6 Annual Meeting, American Academy of Pediatrics. Washington, D.C. Contact: American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

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by Leonardo Mata, M.D.

Intrauterine Growth Retardation: Adaptation or Pathology?
by Joseph B. Warshaw, M.D.

Prader-Willi Syndrome: An Update
by George Bray, M.D.

The Concepts and Mechanisms of Genetic Linkage
by Thaddeus Kelly, M.D.

Genetic Linkage and Endocrine Disease
by Thaddeus Kelly, M.D.

Celiac Disease and Its Effect on Growth
by D.H. Schmerling, M.D.

Pubertal Development in Endurance-Trained Female Athletes
by Alan D. Rogol, M.D., Ph.D.

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Prader-Labhart-Willi Syndrome: An Overview

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Prader-Labhart-Willi syndrome (PLWS), also known as Prader-Willi syndrome, is the most common dysmorphic form of human obesity. The prevalence of PLWS, as estimated from referrals of infants for evaluation of hypotonia, is estimated to be between 1:25,000 and 1:30,000. The features include obesity, short stature, small hands and feet, hypotonia, hypogonadism, and mental retardation. Emotional and behavioral abnormalities, including severe overeating, are common.

Etiology and Genetics

The etiology of PLWS has been the subject of considerable interest for endocrinologists and geneticists. Most cases occur sporadically within families, although recurrence in sibships, as well as concordance in monozygotic twins, has been reported. Prior to the description of cytogenetic abnormalities in PLWS, the risk of recurrence within a sibship was estimated to be 1.4% to 1.6%. Studies of banded karyotypes have shown that many patients with PLWS have chromosomal abnormalities involving the proximal portion of the long arm of chromosome 15. The most common abnormality is an interstitial deletion of band 15q11-13, although other abnormalities involving this portion of chromosome 15, including unbalanced and apparently balanced translocations, have been described.

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Pubertal Development in Endurance-Trained Female Athletes

Alan D. Rogol, M.D., Ph.D.
Associate Editor—Growth, Genetics, and Hormones

Although recreational sports have been available to young women for a number of years, the availability of competitive athletics and other strenuous training routines to females is relatively recent. With a large number of girls, adolescent females, and adult women participating in sports activities, a new set of issues relating to the effects of such training on the reproductive cycle has been raised. The questions below represent a summary of these issues:

- What are the effects of preadolescent endurance-type training on the progression of pubertal development?
- Is menarche delayed by endurance-type training?
- Are physically active girls who mature later better suited for and, thus, more successful in endurance-type events?
- What are the effects of endurance-type training on the reproductive function of postmenarchal adolescents?

Pubertal Process

The normal pubertal process has been extensively reviewed by Tanner et al and will not be restated here, except to note that this pro-

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In This Issue

Prader-Labhart-Willi Syndrome: An Overview

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The reported incidence of cytogenetic abnormalities ranges from 50% to virtually 100%, which may be due to differences in the patient population, the clinical criteria for diagnosis, or the cytogenetic techniques utilized. A study by Niikawa and Ishikiriyama comparing karyotype findings in 12 "classic" PLWS patients with those in 15 patients with less typical disease found that all "classic" patients had deletions involving band 15q11.2, whereas six of the atypical patients had chromosome abnormalities. In another study, Butler et al (1986) demonstrated—surprisingly—that PLWS was diagnosed at an earlier age in cytogenetically normal patients than in patients with cytogenetic abnormalities (5 years, 4 months *v* 9 years, 4 months). They also report other clinical differences in patients with the deletion: lighter hair, eye, and skin color; greater sun sensitivity; and higher IQ. The need for high-resolution chromosome banding to detect some o these deletions and the occurrence of apparent mosaicism in some patients further complicate the interpretation of cytogenetic studies.

Most of the chromosomal abnormalities seen in PLWS, particularly the interstitial deletions of 15q11-12, have occurred in families in which both parents had normal karyotypes. Studies of chromosome markers to determine the parental origin of the abnormal chromosome have shown that it is usually of paternal origin. Butler et al (see Table) found 13 "informative" karyotypes for determination of parental origin of the abnormal chromosome 15; all the abnormal chromosomes were paternally derived and represented de novo deletions.

Chromosomal analyses have proved particularly helpful in assessing hypotonic infants in whom the diagnosis of PLWS is being considered. Such studies have enabled physicians to diagnose PLWS in infants who have not yet become obese. This could lead to earlier intervention to try to control weight gain.

Clinical Features

Despite the developments in the cytogenetic identification of PLWS, most patients are still diagnosed on the basis of clinical findings. The Table summarizes the clinical features as reported in four series of patients. As might be expected, those features by which the diagnosis is made are seen in most, if not all, patients. Apparent differences in the incidence of certain findings could reflect real differences in the patient populations, the clinical criteria used, or the age at diagnosis. Certain features (such as short stature or obesity) may not be apparent in younger patients, whereas other features (such as hypotonia) may become less obvious with age.

Fetal and neonatal hypotonia and feeding problems in the newborn are the earliest clinical features of PLWS and may be indications for chromosome analysis. The feeding problems usually involve an inadequate sucking reflex, sometimes necessitating alternative feeding regimens such as gavage or even gastrostomy. Muscle tone may improve with age, usually late in the first year of life.

The facial features of individuals with PLWS are characteristic and include a narrow bifrontal diameter, almond-shaped eyes with mildly upslanted palpebral fissures, a thin upper lip, and downturned corners of the mouth.

Intelligence and Development

Delay in attaining early developmental milestones and mental retardation are common in PLWS. Part of the early developmental delay can be ascribed to hypotonia, but language and social skills are delayed as well.

The mean IQ for older children is generally around 65, although a wide range has been reported. Butler et al report that the mean IQ of the PLWS patients with a deletion of 15q is higher than the mean IQ of those without the deletion (69.6 *v* 59.2). In a survey reported by Holm (1981), 41% of the patients were normal or in the "borderline" range. Sulzbacher et al (1981) found that the intellectual performance of children with PLWS resembles the performance of children labeled "learning-disabled" more closely than "retarded." This observation may be important in school placement for these children.

Obesity and Diabetes

Obesity is frequently the most obvious presenting sign of PLWS in older children and adults. An increased caloric intake and decreased caloric expenditure and requirements are believed to account for the obesity. Non–insulin-dependent diabetes mellitus (type II) is seen in patients with PLWS and is probably related to the obesity. Five of the first 46 reported patients developed diabetes mellitus, which was diagnosed at ages 11, 12, 16, 17, and 27 years. These patients were described as poorly responsive to insulin, but did not develop ketoacidosis. Ten additional patients from this group had abnormal glucose tolerance but not clinical diabetes. A 2½-year-old patient with PLWS and diabetes has also been described. The incidence of diabetes in patients with PLWS, based on a survey of physicians managing PLWS patients, has been estimated at 7%.

Stature and Endocrine Abnormalities

Although short stature is commonly seen in individuals with PLWS, its basis is uncertain. The average adult height (59 inches for females, 61 inches for males) is well below normal. However, the bone age is usually equivalent to the chronologic age or is only slightly delayed. Studies of growth hormone dynamics show changes similar to those seen in obese controls who do not have PLWS, including "blunting" of the growth hormone response to several pharmacologic stimuli. Treatment

of one patient with exogenous growth hormone did not accelerate the growth rate. Similarly, no increase in linear growth has been observed after treatment with thyroid hormone. Even though a slightly greater thyroid-stimulating hormone (TSH) response to thyroid-releasing hormone (TRH) is seen in PLWS patients than in obese controls, the failure of thyroid treatment to increase growth velocity is not surprising.

Hypogonadism and cryptorchidism are frequently seen in patients with PLWS. Hypogenitalism is seen in both sexes but is more readily noted in males, in whom more extensive studies of gonadal function and histology are available. Histologic examination of the testes has been reported in 12 affected males ranging in age from 4 to 28 years. The prepubertal males have generally shown normal histology for age. In older males, however, the findings have been abnormal, but varied. Sertoli cells have usually been present, but the number and distribution of Leydig cells and germinal cells have varied from patient to patient. Tubules are usually small or atrophic.

The extent of sexual maturation is variable in patients who are not treated with hormones. Menarche may be early, late, or normal, and normal menses as well as oligomenorrhea have been described. Several centers have reported "precocious puberty," usually in females with premature adrenarche. Adrenarche has been reported to occur as early as 5 years of age in PLWS patients, but usually has not been followed by premature menarche. Several patients have been described who had early adrenarche (ages 7 to 8 years), followed by menarche at age 10 years; gonadotropin levels, however, were low. These patients may have a variant form of PLWS, or their premature adrenarche and menarche may be explained by the action of adrenal steroids and the peripheral conversion of adrenal steroids to estrogens. Adrenal steroids could produce "adrenarche" (development of pubic hair and early axillary hair), and peripheral conversion (in adipose tissue) of adrenal steroids to estrogen could cause estrogenization of the uterus and result in

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Table Clinical Findings From Five Groups of Patients With Prader-Labhart-Willi Syndrome

	Hall and Smith (1972)	San Francisco Study (1980)	Bray et al (1983)	Butler et al (1986)	
Number of patients	**32**	**20**	**21**	**21 (D)***	**18 (ND)***
Gestation					
Poor fetal vigor	74%	85%	84%	90%	81%
Breech presentation	40%	22%	38%	35%	24%
Nonterm delivery	43%	43%	33%	55%	77%
Neonatal period and infancy					
Low birth weight (<5 lb)	21%	41%	20%	5%	11%
Hypotonia	100%	100%	100%	100%	100%
Feeding problems	100%	100%	90%	100%	100%
Delayed milestones	97%	100%	90%	100%	94%
Central nervous system					
Mental retardation	97%	100%	100%	100%	100%
Seizures	16%	20%	20%	25%	22%
Personality problems	71%	60%	71%	71%	72%
Growth					
Obesity	100%	100%	100%	86%	88%
Short stature	94%	90%	90%	43%	50%
Delayed bone age	50%	—	90%	—	—
Sexual development					
Cryptorchidism	84%	87%	100%	100%	100%
Hypogenitalism (males)	100%	87%	100%	100%	100%
Menstruation	—	—	33%	43%	25%
Other					
Strabismus	40%	67%	95%	—	—
Small hands and feet	79%	100%	100%	—	—

Adapted from Bray GA et al: The Prader-Willi syndrome: A study of 40 patients and a review of the literature. Medicine *1983; 62: 59-80 .*

**D = deletion; ND = no deletion (of chromosome 15).*

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breakthrough bleeding, which could be misinterpreted as menarche. One patient who was said to have had menarche at 11 years of age was found to have no rise in luteinizing hormone (LH) or follicle-stimulating hormone (FSH) after administration of luteinizing-hormone–releasing hormone (LHRH). Examination of her ovaries following her unexpected death revealed immature organs; there was no evidence of ovulation. It is possible that some of the reports of precocious puberty are correct, but such cases need to be carefully studied and documented.

Basal serum LH and FSH levels in PLWS patients have been low or inappropriately normal, given the low serum concentrations of gonadal steroid hormones. The LH response to injected LHRH in PLWS patients in their second or third decade has usually been subnormal. Response to clomiphene treatment in males has been variable, perhaps related to dosage and duration of treatment. Treatment of a 23-year-old male with clomiphene was followed by maturation of the testes and an increase in serum testosterone and LH. These findings suggest that the pituitary-gonadal axis in PLWS patients can be stimulated by clomiphene treatment to secrete gonadotropins and gonadal steroids. Treating males with chorionic gonadotropin has also stimulated secretion of gonadal steroids in some but not all patients.

Dynamic studies of adrenal and thyroid function have been normal in most patients with PLWS. Cortisol levels reflect a normal diurnal rhythm, but an impaired adrenal response to adrenocorticotrophic hormone (ACTH), as reflected by plasma cortisol measurements, has been reported. A slightly enhanced release of TSH in response to TRH injection has been described. Measurements of circulating thyroxine are usually normal.

Eight available autopsy reports of PLWS patients (ranging in age from 3 to 45 years) have provided little insight into the pathogenesis of the syndrome. Although several of the clinical features and endocrine abnormalities associated with PLWS suggest a hypothalamic problem, examination of the hypothalamus and pituitary by routine methods has failed to show pathologic lesions. Detailed neuroanatomic studies in documented cases of PLWS are needed.

Therapy

Therapy has generally been directed toward dietary restriction and behavior modification, with some encouraging short-term successes. Pipes (1981) reported success in 19 of 24 patients who were managed in an interdisciplinary nutritional management program and followed carefully. In 11 of 12 patients seen before the age of 7 years, prevention of obesity was reasonably successful (for up to nine years in follow-up). Less success was noted in older children who were quite obese before starting the diet/behavior modification protocol. The long-term outlook for this approach to obesity management has yet to be demonstrated.

Surgical procedures (gastric bypass and, less commonly, gastroplasty and jejunoileal bypass) have been used in patients with morbid obesity who could not be managed with diet and behavior therapy. Although there has been some success with gastric bypass, these procedures should probably be reserved for those patients with life-threatening obesity for whom alternative approaches are not successful.

Educational and behavioral needs should be met on an individual basis, with particular attention to the possibility that the patient might be misclassified as "mentally retarded" when "learning-disabled" might be the more appropriate assessment. It is apparent that patients with PLWS should be managed by a coordinated team that can jointly approach their many medical, nutritional, and psychological problems.

Conclusion

Despite the relatively extensive literature that has accumulated regarding PLWS, the physiologic and biochemical basis of the disorder remains unknown. Most of the published information about PLWS consists of small series of patients from a single institution or anecdotal reports of complications or findings in single patients. There is need for coordinated, collaborative prospective studies of virtually every aspect of this condition, particularly the natural history, anatomic findings, and effectiveness of therapy.

Previous reliance on strictly clinical criteria for diagnosis has made interpretation of the literature and comparison of separate studies difficult. The finding of a specific cytogenetic abnormality in many PLWS patients may help to standardize the patient population available for study so that results of collaborative efforts will be more meaningful. Even the isolated

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case report, if well documented, can contribute to our understanding of this condition. Earlier diagnosis by use of cytogenetic analysis of hypotonic infants may allow for more rigorous attention to diet and behavior and could decrease the morbidity and mortality associated with PLWS. The possibility that at least part of the gonadal dysfunction is of later onset, and is perhaps responsive to pharmacologic intervention, suggests that anticipatory treatment might be helpful.

For further information (for patients and their families) please contact:
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Pubertal Development in Endurance-Trained Female Athletes

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cess is usually an orderly one and is marked by a high degree of variability in its onset and completion. Once entrained, however, the variability between stages, although present, is much less pronounced.

Puberty may be considered delayed in girls if they have not achieved breast budding by 13 years of age or if more than five years have elapsed between breast budding and menarche. If a girl has not begun developing breasts by the time she is 13 years old, one should suspect an abnormality in pubertal development and strongly consider further evaluation. Any female over 15 years of age who has not begun pubertal development, and in whom there is no explanation for its absence, must have medical evaluation.

Sociologic and Phenotypic Effects on Athletic Performance

In addition to the impact of the physiologic processes of puberty on athletic performance, one must also be cognizant of sociologic and phenotypic factors that occur with or affect athletic performance. Malina et al substantially broadened the analysis of the interaction between training and puberty in their two-part hypothesis. The first part of their hypothesis suggests that the physical characteristics seen with delayed adolescent maturation in females may be associated with successful athletic performance in selected sports. The later-maturing adolescent girl is characteristically longer-legged, narrower-hipped, more linear in physique, lighter in weight (proportional to height), and relatively leaner than her early-maturing peers.

The second part of their hypothesis relates to socialization. The early-maturing girl is perhaps "socialized away" from competitive sports as she loses her litheness

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and the physical structure that is conducive to athletic success. In contrast, the later-maturing adolescent may not experience the same social pressures or desires to "phase out" sports activities as those entering puberty at early or average ages and may be more motivated to compete athletically.

The Effect of Nutrition and Endurance Training on the Reproductive System

Lower animals, subhuman primates, and humans depend strongly on adequate nutritional intake for the reproductive system to function normally. According to Malina's hypothesis, the timing of the onset of puberty is more closely related to body weight and nutritional status than to chronological age. Studies in humans first implicated a "threshold" body weight and then a "minimum" percentage of body fat as important metabolic signals that determine the onset of puberty in girls. More recent reports suggest that this concept is too simple to explain the physiology of pubertal development, although there is a strikingly significant correlation between the onset of puberty and the attainment of a specific body weight or percentage of body fat.

If the attainment of a specific body weight or a specific fat composition does not explain the physiology of adolescent development, what other signals might be responsible for triggering the maturation of the reproductive axis? Recent studies in the monkey have suggested that certain hormones or metabolic substrates such as insulin, glucose, amino acids, β-hydroxybutyrate, or glycerol act as humoral derivatives of body mass and serve as important cues to the reproductive axis to stimulate the onset of puberty. It has been suggested that changes in the concentrations of these metabolic fuels, which occur during the transition from childhood to adulthood, provide a signal to the neuroendocrine centers that regulate reproductive function. This signal may be correlated with the decreasing sensitivity of the hypothalamic gonadotropin-releasing hormone (GnRH) neurons to the feedback inhibition of gonadal steroid hormones or with the intrinsic maturational events in the brain that occur prior to pubertal development.

In the human, the restoration of cyclic reproductive function in women with secondary amenorrhea is associated with either adequate caloric intake or decreased physical exertion. Similarly, delayed puberty and disruption of cyclic reproductive function in women are associated with inadequate caloric intake and increased physical activity. During starvation, the "nonessential" or potentially detrimental processes, such as gonadotropin secretion, are decreased, while those essential for survival, such as adrenocorticotrophic hormone (ACTH) or thyroxine secretion, are retained. Delay in pubertal development during famine and the restriction of fertility to those few months when nutrition is adequate have been noted in the nomadic Kung San hunter-gatherers who live in the Kalahari desert.

Energy expenditure by itself or in concert with low body weight and low percentages of body fat can delay puberty in adolescent female dancers and other endurance-trained athletes. The effects of exercise on pubertal development must be addressed with respect to the variations described within the majority of children who are not in training.

One can readily assess the impact of exercise upon the onset and progression of the pubertal process in girls because early pubertal development does not confer an advantage in many sports. To the contrary, late pubertal development may be characteristic for females in sports like gymnastics or ballet, in which the prepubertal body configuration and flexibility may confer a professional advantage. Consequently, several investigators have attempted to ascertain if prepubertal or peripubertal exercise and training affect the menarche. In the early 1970s, Malina et al showed that a group of track athletes had a later onset of menses than a group of more sedentary women used as controls. They also found that Olympic athletes, who were presumably more highly trained than other female athletes, had later menarche than high school or college athletes.

Warren and Frisch et al have done intensive studies regarding the onset and progression of pubertal development in groups of young ballet dancers. There are unequivocal data that menarche is delayed by one to three years among these dancers and that secondary amenorrhea is quite prevalent. Warren has carefully distinguished between delayed puberty and adrenarche. She found that the dancers had delayed breast development and menarche, which are mediated by ovarian steroid hormones, but normal pubic hair development, which is largely mediated by adrenal androgenic steroids. In addition, the young women had increased long bone growth, an apparent asset for performing. Although the data are not in question, the underlying mechanism is not clearly defined. Since some of these young women progressed rapidly through puberty or attained menarche when forced to stop exercising (usually due to an injury), and since they incurred no change in body weight or body fat when they stopped exercising, Warren favors the hypothesis that it is the energy drain of training and competition rather than diminished body weight or body fat that is the primary cause of delayed menarche.

Women who trained as swimmers or runners before puberty were evaluated by Frisch et al, who noted delayed menarche when these women were compared to women who began training after puberty. The investigators noted a 0.4-year delay in menarche for ev-

ery one year of prepubertal training. The implication is that puberty was delayed because of the early onset of training. However, it is also possible that the body habitus that is conducive to success at these athletic endeavors is also susceptible to delayed pubertal development.

In theory, endurance-trained female athletes might have a characteristic hormonal profile or characteristic hormonal responses to stimuli. Unfortunately, there are very few data in this area. Consequently, relating delayed menarche (or secondary amenorrhea) to exercise must be done by excluding other causes. A number of conditions, including chronic bowel inflammatory disease and anorexia nervosa, must be considered and ruled out in each endurance-trained adolescent athlete before one can ascribe the hypogonadal state to exercise itself. The evaluation of such patients is outlined in Table 1.

Table 1. Clinical Evaluation of Delayed Adolescence in Girls

History

- Age of onset of breast development
  pubic hair
  menarche
- Family history of reproductive system problems
- Linear growth history (growth chart)
- Central nervous system symptoms or signs (eg, craniopharyngioma)
- Pharmacologic agents (eg, phenothiazines)
- Features of anorexia nervosa
- Exercise habits

Physical Examination

- Height, weight, body proportions
- Status of pubertal development
- Presence of uterus (rectal examination)
- Features of chronic systemic disease
- Anosmia (Kallmann's syndrome)
- Evidence of intracranial disease

Screening Biochemical Tests

- Complete blood count, creatinine, liver enzymes, electrolytes, calcium, urinalysis, erythrocyte sedimentation rate
- Endocrine studies—T_4, prolactin, dihydroepiandrosterone sulfate (DHEAS) to assess adrenarche, bone age, serial luteinizing hormone (LH) concentrations (urinary or serum) over three to 12 months to assess pubertal progression.

Other Tests

- As indicated by suspicious preliminary findings

Cessation of menses is the most obvious effect of endurance training upon the reproductive system. However, more subtle effects, such as luteal phase defects and chronic anovulation, can occur with regular or mildly irregular cyclic vaginal bleeding. A wide range of the prevalence of "athletic amenorrhea" (secondary) has been reported: 1% to 43%, compared to a 2% to 5% incidence of amenorrhea in the general population. This broad prevalence range can be attributed to methodologic limitations. For example, the definition of amenorrhea varies from cessation of menses for four months to cessation of menses for 12 months. In any event, young competitive athletes have noted a much higher incidence of amenorrhea than older women who run recreationally.

Are menstrual cycle changes reversible? Reversibility of gonadal axis defects has been assumed, but not proven. In a single study, two marathon runners experienced amenorrhea during long-distance training after running the marathon. Coincident with decreased training mileage, the menses returned in both. The following observations suggest that secondary amenorrhea related to exercise may be temporary:

- When training is interrupted, long-distance runners and ballet dancers resume menses without change in weight
- Menses return when training intensity is decreased below a "critical level"
- Resolution of amenorrhea occurs in rowers after the end of rowing season
- Normal reproductive function is seen in young girl swimmers 10 years after training.

Although one cannot say with certainty that these natural activities of young women have detrimental longer-term reproductive consequences, there are enough anecdotal data to assure these young women that they are not at great risk for reproductive system dysfunction when they elect to reduce their exercise load. Although fertility is unlikely in these amenorrheic patients, it is prudent for them to employ some form of birth control since "athletic amenorrhea" is not an absolute safeguard against conception.

Even when these women are amenorrheic, the available data frequently do not indicate specific reproducible hormonal abnormalities—the serum estradiol levels are usually within the low normal range and the gonadotropin concentrations are in the mid-range or low-normal range for the follicular phase (although they are probably too low for the relatively low levels of circulating estradiol). The responses to exogenous GnRH are normal.

The possible mechanisms for the apparent hypogonadal state in endurance-trained women are outlined in Table 2 and serve as the framework for our own studies summarized below.

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Reproductive System Function in Amenorrheic Long-Distance Runners: Preliminary Results

My colleagues and I recently undertook a cross-sectional study of highly trained adult female athletes. We reasoned that if we could not define an alteration in hypothalamic-pituitary-ovarian function in this highly selected group, we would have great difficulty in defining alterations in girls and young women whose exercise habits were less strenuous. We also reasoned that similar alterations might delay adolescent development in prepubertal girls who were involved in endurance training. Thus, our preliminary data might, by analogy, give us clues to the alterations in the physiologic pubertal process in these young women athletes.

Certain long-distance runners with secondary amenorrhea or severe oligomenorrhea show an unambiguous decrease in pulsatile luteinizing hormone (LH) secretion, despite mean serum gonadotropin, prolactin, and sex steroid levels that are normal. The reduction in LH pulse frequency is associated with normal or increased pituitary responsiveness to GnRH and intact ovarian estradiol secretion in response to GnRH-induced endogenous LH release. These observations implicate an alteration in the brain's regulation of pulsatile LH secretion (see Table 2).

By analogy, I would consider that endurance-type training in the prepubertal or peripubertal female might delay the normal acquisition of pulsatile gonadotropin secretion that occurs at puberty and maintains the prepubertal condition—few pulsations of low amplitude—for an indefinite period. However, it seems quite clear that the maturational processes in the brain continue naturally during this period of heavy exercise since puberty may be telescoped into a brief period (six to 12 months) when endurance-training adolescents diminish their training schedule. Although one cannot locate precisely the altered portion of the reproductive system, abundant data implicate diminished hypothalamic GnRH release as the cause of "athletic amenorrhea" and delayed pubertal progression in young female athletes who are engaged in endurance training. At present there are no data to support or implicate any of the other mechanisms listed in Table 2 as etiologic factors for the amenorrhea in these runners, although one cannot totally rule out these conditions without further study.

In summary, the limited data available indicate that there may be decreased pulsatile release of GnRH and subsequent diminution of pituitary gonadotropin secretory episodes. The control mechanisms as they relate to energy expenditure and nutrition remain speculative.

Table 2. Possible Mechanisms for Hypogonadism in Endurance-Trained Athletes

Brain Mechanisms
- Altered frequency or amplitude of basal pulsatile gonadotropic secretion
- Loss of estrogenic positive feedback
- Enhanced sensitivity to estrogenic negative feedback

Pituitary Mechanisms
- Impaired synthesis/release of gonadotropins
- Biologically subactive gonadotropins

Gonadal Mechanisms
- Diminished target-organ sensitivity

Other
- Hyperprolactinemia (multiple mechanisms)
- Beta-endorphin hypersecretion
- Variable metabolic clearance rates of gonadal steroid hormones

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IN FUTURE ISSUES

Diarrhea and Growth in Third World Countries
by Leonardo Mata, M.D.

Intrauterine Growth Retardation: Adaptation or Pathology?
by Joseph B. Warshaw, M.D.

The Concepts of Genetic Linkage
by Thaddeus Kelly, M.D.

Genetic Linkage in Endocrine Disease
by Thaddeus Kelly, M.D.

Celiac Disease and Its Effect on Growth
by D.H. Shmerling, M.D.

Prospective Screening for Down's Syndrome Using Maternal Serum AFP

Maternal serum alphafetoprotein (MSAFP) has been used for many years to screen for neural tube defects. Recently, an association between low MSAFP levels and fetal chromosomal anomalies has been observed. It has been postulated that maternal screening for neural tube defects could be used not only to look for high AFP levels, which indicate a risk of a neural tube defect, but also for low values to identify prospectively fetuses with Down's syndrome. In this paper, the physicians responsible for the Connecticut genetics program reviewed their data from MSAFP screening for neural tube defects during the last four years. The normal values of alphafetoprotein must be adjusted for gestational age, maternal weight, and maternal age.

Women 35 years of age and older at the time of delivery have traditionally been offered prenatal diagnosis for chromosomal abnormalities. However, women less than 35 years old have not been offered prenatal diagnosis routinely because of the relatively low risk of having a child with a chromosomal abnormality. These authors suggest that when an inappropriately low MSAFP level is found in women under 35, a second trimester amniocentesis should be offered. They calculate that if their criteria for identifying low MSAFP levels are used, and if women under 35 with low MSAFP levels are offered amniocentesis, one amniocentesis out of 350 would be positive for a chromosomal problem. The present screening policy for chromosomal anomalies in the fetuses of women over 35 years of age is estimated to identify only 10% to 20% of all Down's syndrome pregnancies. Using low MSAFP levels to screen mothers under 35 years of age would be expected to identify an additional 20% to 25% of cases. Thus, the authors suggest that using the combined approach of amniocentesis or chorionic villi sampling in women over 35 plus MSAFP screening with subsequent amniocentesis for those with low values could be expected to identify up to 50% of all children with Down's syndrome prior to 20 weeks of gestation.

Baumgarten A, Schoenfeld M, Mahoney M, et al: *Lancet* 1985;1: 1280-1281.

Editor's comment—*With the institution of maternal screening for alphafetoprotein in most states, it is anticipated that many fetal abnormalities will be identified. This type of program, which is aimed at identifying neural tube defects, will certainly detect a large number of fetuses with anencephaly and spina bifida in the absence of a positive family history. In addition, a number of other abnormalities associated with high alphafetoprotein levels (eg, Turner's syndrome and hydrops) will be found.*

One outgrowth of the MSAFP screening program has been the recognition that a low MSAFP level may also have important diagnostic ramifications since fetuses with Down's syndrome have, on the average, low alphafetoprotein levels in amniotic fluid and maternal serum. The pathogenetic mechanism is unclear, but the potential usefulness of screening is obvious. The costs of such a program may be enormous, but it would appear that if these authors' calculations are correct, the cost:benefit ratio favors this approach.

Growth Without Growth Hormone: Evidence for a Potent Circulating Human Growth Factor

The investigators present a case report of a boy with poor growth and growth hormone (GH) deficiency who, at 4½ years of age, began to grow spontaneously at an accelerated rate (more than 7 cm/yr for more than five years). His bone age rapidly advanced from 3.6 to 12 years and he became massively obese. Repeat GH testing showed inadequate responses to pharmacologic stimuli, whether measured in the immunoreceptor or radioreceptor (IM-9 cell) assay. Somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) levels were within the hypopituitary range when measured by radioimmunoassay.

Laboratory investigation was undertaken to try to determine the etiology of the patient's accelerated growth. Relative somatomedin bioactivity by the embryonic chick pelvic rudiment method was nearly the same as that of a reference pool from normal children. The patient's serum had very great activity in an assay of erythroid progenitor cells (measuring burst-forming units), indicating the presence of a circulating growth factor different from those usually described.

Geffner ME, Lippe BM, Bersch N, et al: *Lancet* 1986;1:343-347.

Editor's comment—*This single case report may provide evidence for the growth observed in some children with intracranial tumors before or after therapy. Some children grow well despite low levels of GH and Sm-C. Many are obese, as is this child. His serum obviously contains a growth factor (at least for erythroid progenitor cells) that is not derived from epithelial, nerve, fibroblast, or platelet growth factors, since these are inactive in the BFU-E bioassay under the conditions employed. It is from the study of such patients and the extraction and purification of the appropriate "growth factor" that therapeutic strategies can be developed for short children and probably for those needing an anabolic but nonandrogenic agent (eg, patients with severe burns or debilitating nutritional disorders).*

Adrenarche and Skeletal Maturation During Luteinizing-Hormone–Releasing Hormone Analogue–Suppression of Gonadarche

The increased secretion of adrenal androgens and the associated early signs of sexual maturation are called "adrenarche." However, during puberty the effects of adrenal androgens upon skeletal maturation are masked by the influence of the more potent gonadal steroid hormones. The investigators have employed a human model to examine the role of adrenal androgen secretion on skeletal maturation. They studied 29 children with central precocious puberty whose gonads were suppressed with an analogue of gonadotropin-releasing hormone factor ($GnRH_a$).

Dehydroepiandrosterone sulfate (DHEAS) levels, an index of adrenal maturation, were constant or increased in an age-expected manner. Ten of the 29 children had DHEAS levels above the normal range for their age. However, premature activation of the adrenal androgen axis did not always correlate with gonadarche; nor did the presence of pubic hair necessarily correlate with DHEAS levels. The increment in bone age over the increment in chronologic age decreased from 1.7 to 0.5, indicating that the $GnRH_a$ induced a return to a prepubertal gonadal steroid environment; this was associated with a slowing of skeletal maturation. DHEAS levels correlated roughly with skeletal maturation rate before and during therapy.

Thus, the data suggest that adrenal androgens may contribute importantly to epiphyseal maturation, although there does not appear to be a strict correlation among bone age maturation, adrenal androgen secretion, and onset of gonadal activity, at least in these patients with premature puberty.

Wierman ME, Beardsworth DE, Crawford JD, et al: *J Clin Invest* 1986;77:121-126.

Editor's comment—*These data confirm and extend previous studies that have suggested the independent control of adrenal and gonadal steroid hormone secretion and the physical signs of pubarche and gonadarche. The data in the younger subjects show that premature activation of the gonadal axis does not necessarily imply premature activation of the adrenal axis. Moreover, the association between increasing levels of adrenal androgens and accelerated rates of skeletal maturation during childhood was confirmed. Those who remained pre-adrenarchial during therapy exhibited the greatest slowing of their skeletal advancement. Thus, adrenal androgens may play an important role in skeletal maturation.*

Testing With GRF (1-29) NH_2 and Somatomedin-C Measurement for the Evaluation of GH Deficiency

A large number of provocation tests are available for evaluating the somatotropic function of the adenohypophysis. The tests are performed with substances and doses that exhibit pharmacologic, but not physiologic, effects. With most tests, it remains unclear whether they directly stimulate the pituitary or the hypothalamus. The detection and synthetic production of growth-hormone–releasing factor (GRF) (1-40) and GRF (1-44) have enabled us to estimate the secretion of growth hormone (GH) in a physiologic and specific way.

Recently, Ranke et al performed similar GRF tests in a large series of patients with growth disorders, administering the fragment (1-29) NH_2 as an intravenous bolus in a dose of one μg/kg. In addition, serum somatomedin-C (Sm-C) levels were determined by radioimmunoassay (RIA). Thirty-eight children with familial short stature, familial tall stature, early normal puberty, or premature thelarche and pubarche served as controls. The usual arginine and insulin tests were normal in an additional 48 children with intrauterine growth retardation (IUGR), constitutional delay of growth and adolescence, dysmorphic dwarfism, or Turner's syndrome. These children were compared to 45 children with growth hormone deficiency (GHD) and abnormal insulin and arginine tests. Prior to these investigations, comparative measurements with GRF (1-40) and GRF (1-29) NH_2 were performed in 11 healthy volunteers. The results of both studies were indistinguishable from each other.

In the control group, the median maximal concentration that was reached was 45.3 ng/ml. The values were distributed logarithmically: ln x ± ln SD was 3.81 ± 0.67. The lowest normal GH value was 10.0 ng/ml. The median maximal values for the other groups were: IUGR, 67.2; constitutional delay, 28.0; dysmorphic short stature, 85.9; and Turner's syndrome, 25.8. Statistically, no difference could be established between and among the various groups. Correlations with age, sex, relative height, and pubertal development were not statistically significant.

In the pituitary dwarfs, the median maximal value was 5.1 ng/ml, but the individual levels varied considerably. In 11 patients, maximal GH levels exceeded 10 ng/ml, but all levels fell below 40 ng/ml. There was no significant correlation between the maximal GH levels after GRF and the peak values after arginine and insulin. However, the correlation between the peaks after GRF and the max-

imal levels reached during deep sleep was positive.

Sm-C levels above 0.4 U/ml in healthy prepubertal children and above 0.6 U/ml in pubertal children were considered normal. Sixteen of 22 prepubertal patients with GHD had both subnormal GH peaks after GRF and low Sm-C levels.

In 12 of 38 controls, the Sm-C concentration was <0.4 U/ml, with normal peak values seen after GRF administration. In 19 of 23 pubertal patients with GHD, Sm-C and GH determinations were subnormal. One of the remaining four patients had a low Sm-C level with a normal peak of GH after GRF; the other three had nomal Sm-C and GH levels. In these latter children, the previously established diagnosis of hypopituitarism certainly should be questioned. Nevertheless, 11 (25%) of 45 GH-deficient patients had GH increases in response to GRF that were within the normal range. Consequently, one has to assume a normal adenohypophysis in these patients and hypothalamic GRF deficiency as the primary cause of the dwarfism in this group. This is in accordance with earlier findings.

Ranke MB, Gruhler M, et al: *Eur J Pediatr* 1986;146.

Editor's comment—*This is one of the largest published series evaluating the GRF test. The authors, among others, confirm the usefulness of the GRF (1-29) NH_2, which appears equally as potent as GRF (1-40) and GRF (1-44). With regard to the pathogenesis of pituitary dwarfism, it appears that a large number (25%) of patients have GHD due to a primary hypothalamic defect. However, it is probable that many more patients might actually have primarily impaired GRF production if the GRF test were to be repeated after several days of priming with GRF. One would not necessarily expect a pituitary gland that has been at rest for many years to respond fully to one dose of GRF. With regard to the diagnostic value of the GRF test, investigation with GRF alone may produce a rather high incidence of false-negative results in patients with GHD. Thus, the combination of GRF and arginine and/or another test, along with the determination of Sm-C concentration, appears helpful. On the other hand, the use of Sm-C values alone entails the danger of obtaining too many false-positive results in patients who may have GHD. Thus, the Sm-C level can be complemented by the GRF test; these tests plus another test for GH sufficiency using a pharmacologic agent, such as insulin or L-dopa, are important in evaluating patients with suspected GHD.*

Role of GH-Releasing Factor and Somatostatin on Somatic Growth in Rats

The investigator studied the role of growth-hormone–releasing hormone (GHRH) and somatostatin (somatotropin - release – inhibiting factor [SRIF]) in affecting growth hormone (GH) secretion and long-term growth in the rat by passively immunizing animals with antisera raised against GHRH and SRIF. GHRH antiserum administration significantly inhibited the normal increase in body weight observed in both young male and female rats as well as in newborn rats. The effects of GHRH and somatostatin antisera administration on serum GH concentrations were studied in neonatal rats. In animals between 1 and 20 days old, GHRH-antiserum administration significantly decreased serum GH concentrations compared with levels in control animals. In animals between 1 and 10 days of age, SRIF-antiserum treatment had no effect on GH concentrations, whereas SRIF-antiserum treatment significantly increased GH concentration in 15-day-old and 20-day-old animals.

Wehrenberg WB: *Endocrinology* 1986;118:489-495.

Editor's comment—*These results confirm that the control of pulsatile GH secretion is through the episodic release of GHRH. Thus, it is not unexpected that those rats treated with GHRH antiserum would grow at a reduced rate; however, no data were presented to determine what organ systems were affected. Both male and female rats showed similar 25% to 30% decrements in weight gain, implying that GHRH is not involved in regulating the sexually dimorphic growth rates. In addition, the antiserum to GHRH was effective from birth, suggesting that neonatal, as well as later, growth is dependent on GHRH secretion.*

In contrast, the passive immunization of neonatal rats with an antiserum to SRIF indicated that it is not until sometime after the tenth day of age that endogenous SRIF can actively regulate GH secretion. Previous investigators have not been able to show biologic effects of SRIF in animals under 5 days of age, so this finding in the present study is not unexpected. Thus, the results suggest that the elevated GH concentrations in neonatal rats are due to hypothalamic GHRH release.

That the rats treated with GHRH antiserum grew at all implies that the pituitary may release GH by a non–GHRH-dependent mechanism, or that some other growth factor(s) is (are) responsible for part of the complex process called growth.

One cannot necessarily transfer results obtained in rats to humans. It would be interesting to ask, however, if the human neonate has the same mechanism for GH release since human neonates have elevated GH determinations during the first few days of life.

MEETING CALENDAR

October 5-10 Fall Meeting. American Physiological Society. Clarion Hotel, New Orleans, Louisiana. Contact: Federation of Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-7010)

October 9-10 9th Annual Current Concerns in Adolescent Medicine. Warwick Hotel, New York, New York. Contact: M.J. Boehme, Associate Director, Continuing Education, Long Island Jewish Medical Center, New Hyde Park, NY 11042 (718-470-8650)

October 9-11 15th Annual Meeting. Child Neurology Society. Boston, Massachusetts. Contact: Child Neurology Society, National Office, Box 486 Mayo, 420 Delaware Street SE, Minneapolis, MN 55455 (612-376-3692)

October 16-18 Pediatric Nutrition Update. San Francisco, California. Contact: Extended Programs in Medical Education, University of California, Room U-569, San Francisco, CA 94143-0742 (415-476-4251)

October 29-30 Update on Endocrinology. The Cleveland Clinic, Cleveland, Ohio. Contact: Center for Continuing Medical Education, the Cleveland Clinic Foundation, 9500 Euclid Avenue, Room TT3-301, Cleveland, OH 44106 (800-762-8137) (800-762-8172 in Ohio)

November 1-6 Annual Meeting, American Academy of Pediatrics, Washington, D.C. Contact: American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

November 2-5 37th Annual Meeting, American Society of Human Genetics. Philadelphia, Pennsylvania. Contact: Gerry Gurvitch, Administrative Director, American Society of Human Genetics, 15501 B Monona Drive, Derwood, MD 20855 (301-424-4120)

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Diarrhea and Its Effect on Growth

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The predominant etiology of diarrhea in the general population of underdeveloped countries is infection by viruses and bacteria. Studies in rural areas clearly suggest an infectious cause. For example, diarrhea initially affects one individual in the family (index case) and then spreads to other family and community members. Infants and toddlers are affected more frequently than older children, adolescents, and adults. The high prevalence of diarrhea in populations with poor personal hygiene and deficient environmental sanitation also points to an infectious cause and is supported by the identification of rotaviruses, *Campylobacter*, enterotoxigenic enteric bacteria, *Cryptosporidium, Shigella, Vibrio cholerae, Salmonella, Giardia*, and other parasites in most patients with diarrhea.

Longitudinal studies of children in deprived ecosystems have documented the significance of diarrheal disease in respect to poor nutrition and growth.[1-3] These studies reveal not only the frequency of diarrhea in infants and young children but also the severity of damage from infectious diseases of the GI tract and their resultant inhibition of good nutrition and normal growth. Many children in Guatemala, Bangladesh, and northeastern Brazil experience from six to nine episodes of diarrhea per year during their first three years of life.[1-3] Most episodes last for a few days and resolve without serious consequences. Other incidents result in considerable losses of fluids and electrolytes or are accompanied by fever, anorexia, and considerable damage to the intestinal mucosa. The worst episodes yield sequelae and permanent damage, such as growth retardation, or result in death.

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Perspectives on Intrauterine Growth Retardation

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Introduction

Fetal growth can be defined in terms of changes in newborn size, organ growth, and maturation, and by the many biochemical adaptations that prepare the fetus for extrauterine existence. Intrauterine growth retardation (IUGR) can result from environmental and genetic influences that limit the intrinsic potential of the fetus to grow, or that restrict growth because of decreases in the amount of available nutrients. IUGR is most commonly defined as a birth weight of less than the tenth percentile at a given gestational age. It is only within the past 20 to 25 years that clear distinctions have been made between low birth weight caused by IUGR and that due to preterm labor. There may be a considerable overlap between IUGR and preterm delivery, which refers to birth at less than 38 weeks' gestation, largely because some of the same risk factors are common to both conditions. Teenage pregnancy, for example, may result in high risk for both prematurity and IUGR. Prognosis of IUGR depends on the underlying condition to a major degree.

Influences on Fetal Growth

A variety of genetic and environmental factors affect fetal growth.[1,2] Genetic factors may be responsible for species or popu-

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Effect of Diarrhea on Growth

Upon detection of growth deficiency or failure to thrive, pediatricians in industrial nations rarely consider infection as the first diagnostic possibility. In these nations, most problems of growth failure due to gastrointestinal disturbances are related to physiologic, enzymatic, immunologic, or metabolic alterations of a noninfectious nature[4] rather than infectious causes.

In developing countries, however, the situation is quite different—particularly in infants who are not breast-fed. Interestingly, most breast-fed children grow very well, even under extreme poverty.[1] By contrast, most children in poor rural and urban areas who are not breast-fed suffer several diarrheal episodes each year, usually resulting in weight loss. Diarrhea-induced weight loss is difficult to correct without prompt and adequate nutritional dietary therapy. Diarrhea often persists in children, even after correction of the infection. This persistence and recurrence of diarrheal episodes generally do not permit catch-up growth. This sequence of events is exemplified by the typical Guatemalan village child whose growth is illustrated in Figure 1.[5] In many rural areas, from 5% to 20% of the diarrheas persist for several days or weeks because of *Shigella* infections. Unfortunately, appropriate antibiotic therapy required for resolution of these infections is not available in most poor rural areas.

Another possible factor in persistent diarrhea is lactose intolerance—a frequent finding, particularly in viral diarrhea during the first year of life. For these infants, diarrhea persists for as long as they are fed cows' milk.

The Guatemalan boy whose growth curve is diagrammed in Figure 1 grew well during the period of exclusive breast-feeding and his growth parameters fell along the 50th percentile of the growth chart of the National Center for Health Statistics (NCHS). When food supplementation was begun at approximately 6 months of age, however, a continuum of diarrheal episodes and weight loss was observed in connection with recurring gastrointestinal and upper respiratory infections. By 1 year of age, the child had experienced several bacterial and viral infections, and altered physical growth was apparent.[6] In addition, infection with parasitical organisms, such as round worms, may also disturb normal growth[6] and may have contributed to alterations in this child.

By age 1, the child was distinctly wasted. The encounter with a variety of viruses, bacteria, and parasites continued, and for one year the child remained wasted and at risk of developing severe protein-energy malnutrition or of dying. The possible metabolic and hormonal disturbances in children under such circumstances—who represent the majority of cases in deprived villages and slums—have not been established.

Field studies show that diarrhea adversely affects nutrition and physical growth.[7-9] Figure 2 illustrates the relationship of growth retardation to diarrheal episodes in two Cauque children. Each recorded episode of diarrhea of known or unknown etiology coincided with an arrest in linear growth. These arrests were of shorter duration and negligible consequence during the first months of exclusive, intensive breast-feeding; upon weaning, however, the magnitude of arrested growth was more marked, often extending for several weeks or months. The effect was even more pronounced in the child with severe fetal growth retardation, as seen in the right side of Figure 2.

These cases demonstrate the prolonged effects of inadequately treated diarrhea and the lack of rapid catch-up growth because the child is repeatedly stricken with infectious episodes. Eventually, the cumulative effects of these episodes (with the additive effects of otitis media, acute respiratory infections, or exanthemas of early childhood) result in a markedly diminished growth rate. In the study village, virtually all children showed some degree of stunting by age 2 years. Cohorts defined by birth weight or by fetal maturity

Figure 1. Typical growth pattern of a Guatemalan village child. Note the drop-off in growth at about five months when the child was weaned from breast milk.

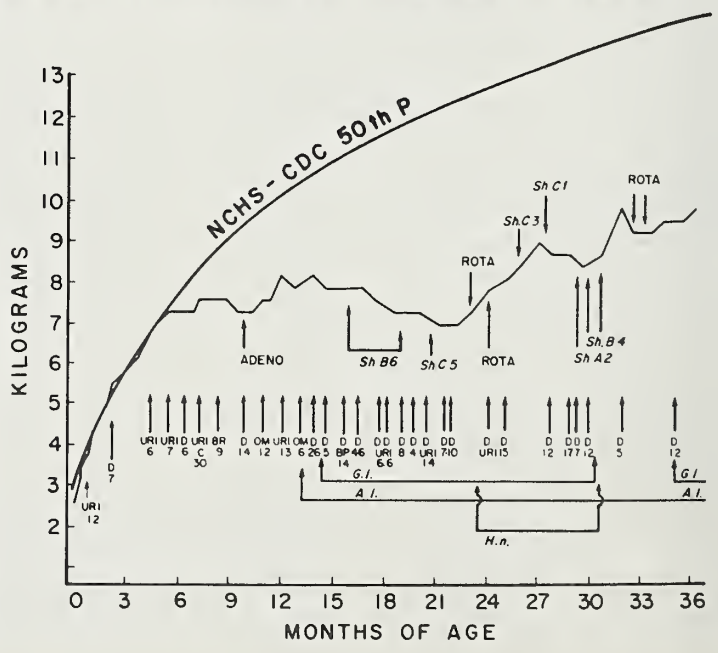
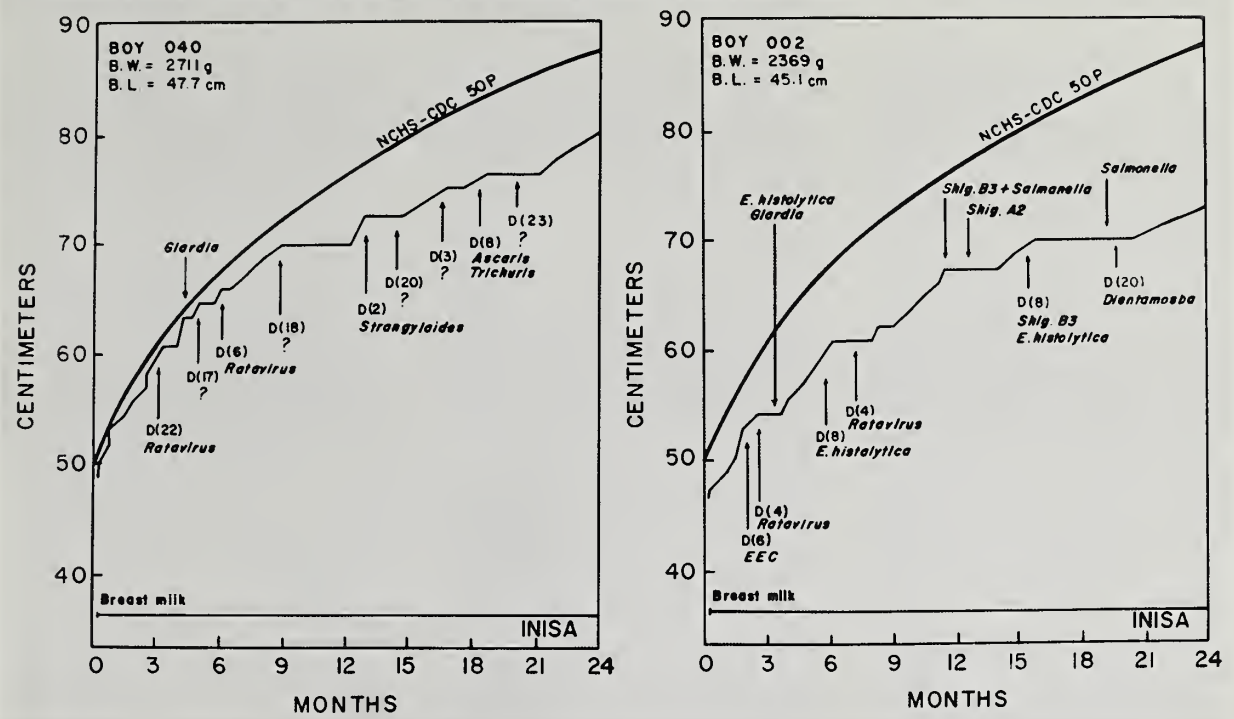


Figure 2. The relationship of drop-offs in growth to infectious diseases is clearly illustrated in these two children. The effect is more pronounced in the pattern on the right of a child with severe fetal retardation.



exhibited a positive correlation between intrauterine growth and poor postnatal physical growth.[1,7] The greatest impact is delivered by the adverse microbial environment of underdeveloped countries.[1,5] Overall growth deficit and much of the wasting reported in children throughout the world is probably the result of repetitive diarrheal diseases and other infections, which severely aggravate an already marginal or poor nutritional intake.

A long-term study, conducted in the poor, rural population of Costa Rica, a country in transition, demonstrated that growth was significantly improved as better sanitation was developed.[9] Although food supplementation during weaning frequently was not improved, the less intense infectious environment resulted in very low rates of enteric infection and diarrheal disease.[10] While growth failure was occasionally observed, it was the exception and was attributable to organic disorders or child neglect, as is the case in industrial nations.[9]

Infectious Diarrhea Induces Malnutrition

Diarrheal diseases are the most important inducers of malnutrition worldwide, because they alter nutrition—and growth—through reduced food intake, disturbed digestion and absorption, impaired use of nutrients, and other metabolic alterations. Each episode has a varying impact on the host economy and nutrition, even when there is no limitation in food availability.[7] Diminished intake of food during diarrheal episodes is often substantial, especially among infants and toddlers. There are two predominant causes: anorexia and restriction dictated by traditions, beliefs, and taboos. In the latter case, the mother or other caretaker suppresses the food intake for days or even weeks, in the belief that food perpetuates the diarrhea.

Anorexia appears to be the most significant reason for decreased food consumption. It is triggered by interleukin 1 (previously known as leukocyte endogenous mediator) and by cachectin (tumor necrosis factor), hormone-like substances released by macrophages and monocytes under the stimulus of infections or other stress. A manifestation of the "generalized acute-phase metabolic response," anorexia occurs regardless of the type, severity, and localization of infection.[11] Most foods are rejected, although breast milk is least so. The intensity of anorexia does not always correlate with the kind or severity of illness, and a child may become anorectic even with a common cold or mild diarrhea. The effect may last a few hours or extend for days or weeks. As much as 20% to 70% of the available food may be wasted or uneaten during bouts of diarrhea.[7]

Microbial action also increases intestinal secretion and lysis of cells in villous tips by rotaviruses, for example, or by stimulation of cyclic AMP and cyclic GMP by bacterial enterotoxins such as *Escherichia coli*. If repetitive losses are not corrected by rehydration and other therapies—

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often unavailable in villages in poor countries—they contribute to malnutrition. Hypersecretion also can be induced by bile and fatty acids, hormones, and neurotransmitters, and by greater calcium cell permeability induced by mediators.[12] Agents such as *Giardia* adhere to the surface of enterocytes, while others such as *Cryptosporidium* lodge under the microcalix but outside the cytoplasm. Some parasites multiply within epithelial cells and in the lamina propria, causing inflammation and bleeding (*Shigella*), or burrow in tissue, eliciting a granulomatous response (*Entamoeba*), or they reach lymph and blood vessels, resulting in sepsis (*Salmonella*).

These infections may generate profuse loss of water, electrolytes, cells, and nutrients, reducing the host to a state of acute malnutrition. Patients, especially infants and young children, may lose 10% or more of their body weight within hours, and may die if shock and dehydration are not promptly corrected. Cells, plasma, amino acids, lipids, vitamins, and hormones may be lost with injury to intestinal mucosa. The dysentery diarrheas are more damaging because they often are accompanied by a protein-losing enteropathy,[13] and exhibit toxic manifestations with weakness and prostration and high mortality.

As with other infections, diarrhea is accompanied by anorexia and fever, breakdown of muscle protein, discharge of insulin and glucagon, mobilization of leukocytes, and sequestration of zinc and iron. Vasoactive intestinal polypeptide (VIP), which inhibits the peristaltic reflex, and other gut hormones (motilin, enteroglucagon, and neurotensin) are increased or decreased during diarrhea. Prostaglandins are increased in diarrhea, including the mild forms seen in toddlers.[14]

Conclusion

Infectious diseases, and diarrhea in particular, are the main determinants of wastage and stunting of growth in children in underdeveloped countries. Nations that are able to diminish the incidence of diarrhea and other infections clearly exhibit a secular change in growth and height of children, as observed in Chile, Costa Rica, and other countries in rapid transition.[5,15,16] Children with no or fewer infections have better appetites, and their healthy parents provide better care. In turn, society benefits because of better use of available resources and increases in production. This might explain, in part, why certain very poor areas, which remain basically poor and consume minimal food, exhibit a remarkably good health condition. One example is the State of Kerala in India.[16]

Equally interesting is the observation that in some undeveloped countries, provision of food supplementation or food distribution centers has been unsuccessful in combating malnutrition. In particular, this is especially noticeable in the continuing presence of poor sanitation, which leads to diarrhea and other infectious diseases. As part of any major policy to prevent malnutrition in underdeveloped countries, attention must be directed toward the control of infectious diarrhea. Only in this way can malnutrition and growth failure be prevented.[5,17]

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Perspectives on Intrauterine Growth Retardation

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lation differences in size at birth. Mean birth weight in human populations can range from 2,400 g in New Guinea to 3,880 g in American Indian populations. Although some of these differences can be explained by factors such as nutrition and maternal size, it is likely that ethnic differences in birth weight occur regardless of socioeconomic status. Males weigh an average of 150 to 200 g more than females at birth. This difference occurs in late gestation and may be related to the testosterone produced by the male gonad, but this has not been proven.

Hormones are important for fetal maturation and for many of the adaptive events that prepare the fetus for extrauterine existence. Insulin appears to be the principal growth hormone for the fetus. Other classic hormones appear to influence specific organ development rather than fetal size. For example, testosterone induces virilization of the genitalia, glucocorticoid influences lung maturation, and thyroxine modulates central nervous system development. With the exception of somatomedins, other putative growth factors, including epidermal growth factor, nerve growth factor, and transforming growth factors, are of likely importance in regulating organ growth and differentiation without greatly influencing newborn size. Somatomedins are present in and synthesized by a variety of fetal tissues, and umbilical cord levels of somatomedin-C have been correlated with birth weight.

Genetic and/or chromosomal disorders can profoundly alter fetal growth, with the degree of growth failure reflecting the specific defect. Growth retardation is a major feature of Down's syndrome, trisomies 13 and 18, and Turner's syndrome. Intrauterine infections may be responsible for as many as 10% of cases of IUGR and should always be considered in the evaluation. IUGR is commonly seen as part of the symptom complex caused by toxoplasmosis, congenital syphilis, rubella, cytomegalovirus, and herpes simplex (the TORCH organisms). Recently, IUGR-associated malformations, including microcephaly and craniofacial abnormalities, have been described in newborns with an AIDS-related embryopathy.[3]

Drugs and chemicals causing IUGR include classic teratogens, such as antimetabolites, as well as common therapeutic agents such as phenytoin, trimethadione, and warfarin. Heroin addiction, cigarette smoking, and heavy alcohol use are also commonly associated with IUGR. More than 50% of infants born to mothers who drink heavily will be abnormal. In one study, the incidence of IUGR was 7% in babies whose mothers were light-to-moderate drinkers and 27% in those whose mothers were heavy drinkers.[4] Cigarette smoking is a powerful determinant of IUGR and results in a birth weight deficiency of 150 to 250 g.[5] This is most likely related to the combined effects of smoking on maternal appetite, uteroplacental blood flow, and maternal blood levels of carbon monoxide that further impair oxygen delivery to the fetus.

The terms "proportionate" and "disproportionate" have been used to distinguish IUGR newborns with decreased growth potential from those with restricted growth due to impairment of maternal nutrient delivery. Fetuses and newborns with decreased nutrient supplies exhibit disproportionate growth because of a relative sparing of brain growth, whereas congenital infections or genetic diseases that restrict growth potential result in proportionate or symmetrical growth retardation. These patterns of growth can be detected in utero and may indicate the underlying condition that ultimately results in IUGR. Poor maternal weight gain and fundal growth should alert the obstetrician to the likelihood of IUGR so that ultrasonography can be utilized to follow fetal growth parameters such as the biparietal diameter or the relationship of the head size to the body size (which can be used to identify proportionate or disproportionate fetal growth in utero).

Fetal Malnutrition

Fetal malnutrition is the most common cause of low birth weight. It can result from maternal malnutrition or from failure of the fetal circulation to deliver adequate substrates to the fetus generally because of maternal diseases that restrict uteroplacental blood flow. Conditions resulting in decreased uteroplacental blood flow include toxemia of pregnancy and maternal hypertension secondary to chronic renal disease. Fetuses in multiple pregnancies may exhibit restricted growth because of failure of the uteroplacental unit to provide optimal nutrition to more than one fetus in the uterus. The smaller twin of a monozygotic pair frequently exhibits IUGR because of arteriovenous communications within the chorionic plate that can severely compromise blood flow to one twin. IUGR is also observed in infants born in high-altitude regions and in those born to mothers with cyanotic congenital heart disease, presumably because less oxygen is available in both instances.

Maternal Regulation of Growth

Walton and Hammond[6] reported that foals of Shire horses bred with Shetland ponies reflected the size of the mother. Shetland/Shire crosses born to a Shire mare were the size of normal Shire foals, whereas the foals born to the Shetland dam and the Shire cross were the size of the normal Shetland foal. Similar data in other species, including humans, suggest that constraints on fetal growth are imposed by the maternal uterine environment. In human pregnancies, fetal growth is generally not affected by the number of fetuses prior to the 26th week of gestation. After 27 weeks of gestation, however, the growth rate is slowed for triplets; the rate slows after 30 weeks for twins. Uteroplacental constraints may even become op-

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erative in singleton pregnancies when a weight of about 3,000 g is achieved regardless of the number of fetuses.

The Dutch famine of 1944-45 resulted in a mean birth weight reduction of about 300 g.[7] This effect was observed primarily when the period of starvation occurred within the last trimester of pregnancy. Behavioral testing and IQ performance data did not reveal any deficiencies when the population at risk was studied more than 20 years later.

IUGR caused by malnutrition may be multigenerational. In a marginally nourished rat colony maintained over nine generations, maternal weights and newborn sizes were markedly reduced when compared with those in normally nourished controls.[8] With reinstitution of normal nutrition after five generations of marginal nutrition, it appeared that more than one generation of good feeding was necessary to correct the deficits.

Clinical Evaluation of IUGR

Evaluation of the newborn with IUGR begins with measuring length, weight, and head circumference and plotting the results on standard growth charts to determine if the pattern of growth is disproportionate or proportionate. The Lubchenco charts are most commonly used although they may underestimate IUGR as compared with other standards. A careful assessment of gestational age should be made for all infants. Accurate dates can be confirmed by ultrasound examination of the fetus in early pregnancy or estimated less precisely by the Dubowitz exam immediately after birth.

Infants with nutritional IUGR have a scrawny, wasted appearance because they have so little subcutaneous fat. Many of their problems are associated with decreased metabolic reserves. These infants are at increased risk for asphyxia and meconium aspiration; therefore, when IUGR is detected antenatally, there should be appropriate monitoring and careful planning concerning the mode of delivery.

Newborns with IUGR are also at increased risk for hypoglycemia and polycythemia. Hypoglycemia is probably due to low fuel reserves and a decreased capacity to carry out gluconeogenesis. Polycythemia occurs in response to the increased erythropoietin levels secondary to relative intrauterine hypoxia. Chronic intrauterine hypoxia may also result in persistent pulmonary hypertension with marked right-to-left shunting because of abnormal thickening of the small pulmonary arterioles in the hypoxic fetus. These are primarily problems of the nutritionally growth-retarded newborn. Those with IUGR secondary to congenital infection and/or genetic disorders are less likely to develop these complications.

Fetal Adaptation in IUGR

While serious pathology may clearly be the consequence of markedly reduced uteroplacental blood flow, the majority of infants with nutritionally based IUGR have normal development and do not show significant differences in IQ or neurological scores when compared with normal newborns.[9] A strong case can be made that many of the features of nutritional IUGR represent fetal adaptation to a restricted nutrient environment rather than a pathologic condition.[10] Those fetuses with sufficient time to adapt to compromised nutrition may maximize their prospects for a favorable outcome.

In such infants, brain growth is spared because of a redistribution of fetal blood flow. A smaller overall fetal size may reduce substrate and oxygen needs to what can be provided by an impaired uteroplacental circulation. A redistribution of blood flow to the head supports brain growth and head circumference at the expense of both weight and linear growth. Increased blood flow to the brain associated with decreased blood flow to the viscera increases the ratio of head circumference to abdominal circumference; this ratio can be measured in utero with ultrasound and thus identifies disproportionate IUGR antenatally. Vasopressin released in response to oxygen and/or nutrient deficiency is a likely mediator of increased blood flow to the brain. Polycythemia exhibited by these infants can also be viewed as an adaptation that results in an increase in the capacity of the blood to carry oxygen to the organs and tissues of the growth-restricted fetus.

Finally, severe nutrient restriction appears to be associated with accelerated maturation. Data from experimental animals and in humans suggest a lower incidence of hyaline membrane disease in fetuses with IUGR, which may increase survival if the fetus with IUGR is born prematurely. The infant with IUGR may therefore represent a successful adaptation to a substrate-deficient intrauterine environment. Those infants with IUGR who have sufficient time to adapt to a substrate-deficient intrauterine environment may be at lower risk for serious hypoxic injury that may occur in large fetuses born at term but subjected to acute uteroplacental compromise at the time of delivery. In other words, smaller may be better.

If maternal constraints on fetal growth can be viewed as an adaptation in the IUGR pregnancy, questions can be raised about the effects of intervention programs designed to increase fetal weights. This may potentially cause an adverse outcome in chronically malnourished populations that have already adapted to malnutrition and a constrained uterine environment. Careful evaluation and follow-up of such intervention programs are necessary.

In summary, adverse genetic and environmental influences can impose severe constraints on growth. IUGR resulting from congenital infection, genetic or chromosomal defects, and/or drugs and other environmental insults is likely to be associated with long-

term developmental disability. Fetuses with IUGR secondary to intrauterine nutritional deprivation may have more favorable outcomes due in large part to adaptations such as decreased fetal size with sparing of brain growth, mild polycythemia, and enhancement of pulmonary maturation. In many such infants, IUGR is an advantageous adaptation rather than a pathologic condition.

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IN FUTURE ISSUES

The Concepts and Mechanisms of Genetic Linkage
by Thaddeus Kelly, M.D.

Genetic Linkage and Endocrine Disease
by Thaddeus Kelly, M.D.

Turner's Syndrome
by Judith G. Hall, M.D.

Directory of Resource Groups for Patients with Endocrine and Genetic Disorders

Letter to the Editor

Russell-Silver Syndrome

In Vol. 2, No. 2 of *Growth, Genetics, and Hormones,* an article by Saal et al entitled "Reevaluation of Russell-Silver Syndrome" was abstracted. The Editor's Comment on that abstract prompts this letter.

One of the reasons that the Russell-Silver syndrome is heterogeneous is that *there is no such thing as the Russell-Silver syndrome.* Dr. Russell and Dr. Silver, in their original reports, described two entirely different syndromes. It is a mistake to combine the two and perpetuate the combination. I have mentioned this to Dr. Alex Russell, who agrees. Dr. Silver even described increased urinary gonadotropins in his patients. I point this out to our house staff when they refer such a patient to our clinic. In my experience, most of the patients referred to me for dwarfism, triangular facies, and intrauterine growth retardation fall into the "Russell" category. I have yet to see a patient with increased gonadotropins at a young age in the hemihypertrophy syndrome described by Silver. I continue to be a splitter instead of a lumper.

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Dr. Blizzard's Comments

Dr. Green's letter is in accord with the article written by Saal et al and published in the *Journal of Pediatrics* 1985;107:733. These authors stated that the Russell-Silver syndrome is a heterogeneous entity. Dr. Green would say it is not an entity at all. Undoubtedly, many would agree with Dr. Green. I have asked Dr. Silver to respond and his comments are listed below.

Dr. Silver's Comments

The confusion about the Silver-Russell syndrome will undoubtedly continue until the specific etiology(s) of the syndrome has (have) been defined and/or a specific diagnostic laboratory test is available. Although the heterogeneity of findings suggests that multiple etiologies may be involved, there is no concrete evidence that this is so.

The Silver-Russell syndrome certainly fits the definition of a syndrome: "the sum of signs of any morbid state; a set of symptoms occurring together" (Dorland). As with most other syndromes, not every child with the Silver-Russell syndrome has every finding. However, the combination of all or most of the findings of congenital short stature continuing into childhood, asymmetry involving various parts of the body, triangular facies, clinodactyly, cafe-au-lait areas of the skin, syndactyly of the toes, and elevated gonadotropins (as first described by me in 1953, and in subsequent publications, and by Russell in 1954) occurs with sufficient frequency to be considered a specific syndrome with one or more etiologies.

Originally, the syndrome was known as the Silver syndrome in this country and the Russell syndrome in Europe. More recently, it has been termed the Silver-Russell syndrome or the Russell-Silver syndrome. Hopefully, Drs. Green, Saal, and others will soon provide us with the information that will permit us to make etiology-based diagnoses. Until then, I believe it is reasonable to continue using the names that have historically been assigned to what appears to be a single clinical syndrome with a characteristic phenotype.

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The address given for the Prader-Willi Syndrome Association in Volume 2, Number 3 was in error. The correct address is: 5515 Malibu Drive, Edina, Minnesota 55436. The phone number is 612-933-0113. Marge A. Wett is the Executive Director.

Short Stature in Anorexia Nervosa Patients

In following 104 patients with anorexia nervosa, the authors found 85 suitable for comparison with 85 age-matched controls. As seen in the Table, a large percentage of the anorexic patients were short.

Information was available regarding parental heights for 35 patients. The mean actual height was at the 34th percentile, compared to a mean expected height at the 48th percentile, based on calculations of parental heights. Twenty-six females were postmenarchal, permitting comparison with the adjusted mid-parental height (Tanner scale). Nine had evidence of growth impairment and could not be classified under "familial short stature" by this method.

The patients' age at onset of anorexia ranged between 10 and 22 years. Symptoms first appeared an average of 12.9 months before seeking therapy, and the mean weight loss was 29 pounds (25% of total body weight). Of great importance in considering the etiology of the short stature is the fact that 80% developed anorexia after menarche, with symptoms of onset occurring more than one year postmenarche in 61%.

Table Height-Related Statistics in Study Participants

Height percentiles	Anorexic patients n	Anorexic patients %	Controls n	Controls %	Expected, %
<5	12	14	1	1	4
5-9	3	4	4	5	5
10-24	28	33	16	19	15
24-49	22	26	28	33	25
>50	20	23	36	42	50

The conclusion is that some factor(s) other than malnutrition may account for the fairly high incidence of short stature. Possibly, there is a pathophysiologic factor producing short stature and, subsequently, anorexia. Patients with anorexia sometimes exhibit several indications of a hypothalamic abnormality affecting thyroid, gonadal, and adrenal function. The authors state that excessive somatostatin production cannot be excluded.

Nussbaum M, Baird D, Sonnenblick M, et al. *J Adolesc Health Care* 1985;6:453-455.

Editor's comment—*These data are not only important but also provocative, since they are unexplained within the context of current knowledge. Most patients with anorexia might be expected to have growth failure secondary to malnutrition. In the majority of these patients, growth retardation preceded malnutrition. Growth hormone levels are increased in most patients with anorexia, although IGF-I values are low, as is expected with starvation. We do not know whether GH and IGF-I levels are normal before the onset of anorexia. If available, these data might provide insight regarding the etiology of anorexia nervosa.*

Furthermore, could these patients have hypercortisolism long before the anorexia begins? (See the review of the endocrine symposium on neuropsychiatric disorders, reported by Dr. Lifshitz in this issue.) If present, hypercortisolism could account for the growth retardation.

Hypercalciuria, Hyperphosphaturia, and Growth Retardation in Children With Diabetes Mellitus

The authors evaluated 157 diabetic children, 6 to 16 years of age, with insulin-dependent diabetes mellitus (IDDM) from 0.2 to 14 years. Eleven percent of the 157 subjects were shorter than would be anticipated, as assessed by comparison with the controls. Increments in height became smaller with the duration of IDDM and differed significantly from controls when IDDM had been present for more than seven years.

Growth retardation correlated with increased calcium and phosphorus excretion (as reflected by increased Ca/Cr and P/Cr ratios) and with poor control of IDDM (as evidenced by glycosylated hemoglobin assays). Hypercalciuria was *not* correlated with increased serum calcium or other evidence of bone calcium mobilization. Hypercalciuria is reportedly caused by hypophosphatemia, and there was an inverse relationship between serum phosphorus and an increase of urinary P/Cr and Ca/Cr. Renal disease could not be demonstrated as a cause of increased Ca and P excretion when it occurred. The urinary loss of Ca also correlated inversely with plasma glucose at the time of urine collection. The increased urinary phosphorus appears to result from competition between glucose and both Ca and P for renal tubular reabsorption. There was some evidence of hypercalciuria as a renal response to functional phosphorus deficiency.

The authors conclude that the higher incidence of short children with IDDM is primarily associated with poor metabolic control, but the specific mechanism(s) of impaired growth is (are) not well defined and may not be due to a single cause.

Malone JI, Lowitt S, Duncan JA, et al. *Pediatrics* 1986;78:298.

Editor's comment—*This study is very well done and carefully analyzed. The authors speculate that phosphorus supplementation might be beneficial. Further studies are certainly indicated to elucidate the causes and results of hypercalciuria and hyperphosphaturia, which are frequently seen in patients with poorly controlled IDDM. (See Harrison's article in* Growth, Genetics, and Hormones, *vol. 2, no. 2.)*

First Trimester Prenatal Diagnosis: Three Reports

Prenatal diagnosis of severe congenital diseases and malformations, which permits selective termination or altered management of affected pregnancies, has become an accepted part of modern medical practice. In the 1970s, amniocentesis and real-time ultrasound evaluation of the fetus during the second trimester were introduced for prenatal diagnosis. In the early 1980s, first trimester sampling of the chorionic villus (the fetal part of the placenta) was developed as an alternative modality for prenatal diagnosis. By the end of 1985, sampling procedures of more than 1,000 chorionic villi had been performed for prenatal diagnosis during the first trimester in ongoing pregnancies.

The article by Jackson in *Seminars in Perinatology*[1] reviews the technique and the indications for first trimester chorionic villus sampling. The technique involves localization of the placenta with ultrasound, and the vaginal removal (by suction under ultrasonic supervision). The test is most easily and safely done between the beginning of the 9th week and the end of the 11th week of gestation. Chromosomal, DNA, and most biochemical assays can be done on chorionic villus material, and the results of such testing are usually available within the first trimester.

The safety and accuracy of chorionic villus sampling have been established by the Internal Chorionic Villus Sampling (CVS) Registry, which was established by Jackson et al two years ago.[2] It is now clear from these data that the incidence of significant complications after CVS is less than 5%. In institutions with experience in the technique, the miscarriage rate after CVS is between 2% and 4%. The background spontaneous abortion rate is approximately 2% or 3%. Thus, additional risk of CVS-caused miscarriage seems small and is probably in the range of 1%. Separation of fetal from maternal tissue is extremely important for accurate CVS results. One complication that has been observed is a higher rate of chromosomal mosaicism in chorionic tissue than in amniotic tissue.

Transabdominal CVS has recently been described by Smidt-Jensen et al.[3] It may be that this technique will avoid or minimize occurrence of infection, which has occasionally been seen in vaginal sampling.

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Editor's comment—*There are several advantages to first trimester prenatal diagnosis. These include safety for the mother if termination of pregnancy is deemed necessary and a chance to confirm results by second trimester amniocentesis, if appropriate. Earlier testing is also easier to handle psychologically for most families.*

Since prenatal diagnosis is available and since it can be applied to detect many types of growth problems, physicians should be aware of these new advances and the availability of first trimester diagnostic techniques.

Influences in Child Growth Associated With Poverty in the 1970s: An Examination of Hanes I and Hanes II, Cross-Sectional U.S. National Surveys

The association between poverty and growth deficits in children has been reported in developing countries as well as in the United States. In this study, a sample population of 13,750 black and white children aged 1 to 17 years was taken from the Health and Nutrition Examination Surveys, HANES I (1971-1975) and HANES II (1976-1980). These were employed to examine the associations between height, weight, triceps skinfold thickness, subscapular skinfold thickness, and dietary intake measures. The poverty index ratio (PIR) was used to define the poverty threshold. This index represents a more specific measure of poverty than income by including family size and composition, sex of head of household, farm/nonfarm residence, and the current Consumer Price Index. The PIR is widely used by the U.S. Government.

Overall, children above the poverty threshold were taller, heavier, and fatter than children in families living below the poverty level. Specifically, on the average, poor children were 1.3 to 1.9 cm shorter, 2% to 3% lighter in weight, and 3% to 8% leaner (by skinfold measurements) than children above the poverty level. An interesting finding was that there were no reported differences in energy consumption and macronutrient intakes between the two groups. However, a trend toward improved growth among the poor children was noted between the time of the HANES I (1971-1975) and HANES II (1976-1980) surveys.

Jones DY, Nesheim MC, Habicht JP. *Am J Clin Nutr* 1985;32: 714-724.

Editor's comment—*This study suggests that caloric intake* does not *appear to play a role in the growth failure reported among poor children. Both groups of children consumed equal diets, yet children who were below the poverty threshold were smaller in both weight and height, and had less reserve fat as measured by skinfold thickness than children above the poverty threshold. Other factors that may be associated with poverty, such as more frequent infections, insufficient medical care, and poor sanitation, may have had a negative influence on the growth of the children below the poverty threshold. The authors, however, do not discuss these concerns as they relate to growth.*

Special Report: The Endocrine Society Symposium on Endocrinology of Neuropsychiatric Disorders—June 25-27, 1986, Anaheim, California

Fima Lifshitz, M.D.
Associate Editor—Growth, Genetics, and Hormones

The symposium dealt primarily with the interrelationship of nutrition, neuropsychiatric disorders, and endocrinology. Dr. John E. Morley of the University of California at Los Angeles pointed out that many peptide hormones are involved in the control of human eating behavior. For example, cholecystokinin-8 has been called a satiety factor because of its ability to decrease feeding and delay gastric emptying through vagal activity. Dr. Morley also noted that glucagons, somatostatin, bombesin, calcitonin, naloxone, and other opioid antagonists act centrally as satiety factors. Corticotropin-releasing factor is also a potent anorectic agent. Peptides that enhance feeding behavior include the endogenous opioids, pancreatic polypeptide, galinin, growth-hormone-releasing hormone, and neuropeptide Y (bulimin).

Dr. Michelle P. Warren of St. Luke's-Roosevelt Hospital, New York City, discussed endocrine changes associated with anorexia nervosa. Dr. Warren stated that the incidence of anorexia nervosa appears to be increasing. It afflicts between 0.5% and 1.0% of white adolescents who are in the mid-socioeconomic group. There is a 6% concordance in incidence among monozygotic twins although the reasons for this are poorly understood. The peak age of onset is at about 12 to 13 years of age. For some unexplained reason, anorexia occurs more often in girls with scoliosis. The disorder is very rare among blacks and among men (the male-female ratio is 1:9). However, anorexia nervosa occurs in males who are training for competitive athletic activities and are restricting their food intake. Between 5% and 20% of professional ballet dancers can be classified as patients with anorexia nervosa.

The endocrine changes seen in anorexia nervosa appear to be adaptive phenomena and are similar to those seen in starvation. These include lower levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and decreased pulsatility of LH over a 24-hour period. The pulsatility pattern reverts to that seen in prepubertal subjects. There is also increased secretion of endogenous opioids, but administration of naloxone restores normal LH secretion in only a small number of patients. Thyroid function resembles that in the "euthyroid sick syndrome," with increased 3:3′, 5′ triiodothyronine concentrations and decreased 3, 5, 3′ triiodothyronine secretion. This reduces the metabolic rate and decreases muscle catabolism. Hypercortisolism often occurs because of

Special Report: National Foundation-March of Dimes Clinical Genetics Conference on Muscle and Its Disorders—June 8-11, 1986, Philadelphia

Judith G. Hall, M.D.
Associate Editor—Growth, Genetics, and Hormones

The National Foundation-March of Dimes has reinstituted the clinical genetics conferences that were so successful in the 1960s and 1970s. The earlier conferences focused on the delineation of birth defects. However, because of advances in molecular genetics, developmental genetics, and clinical genetics, a new format became desirable. The new March of Dimes clinical genetics conferences are aimed at providing a better understanding of a particular organ system. At this year's conference, the subject was muscle. Clinical and basic research dealing with normal and abnormal muscle differentiation, muscle biochemistry, and muscle function was presented, allowing clinicians and researchers to learn from each other's work.

Sir Andrew Huxley convened the conference with a historical overview of muscle disorders. Several presentations on molecular research related to the actin and myosin genes followed. Not only have these genes been mapped and their differences described, but the progressive switching on and off during development and in different tissues is becoming well defined. The mapping of specific genes that are tightly regulated during embryologic and fetal development was clearly outlined at the meeting. Much of this work has been done in culture of muscle cells, but there seemed to be correlations in different animal model systems and in muscle from various sites of the body.

The clinical aspects of well-defined muscle disease, both dystrophies and metabolic disorders, were reviewed. However, a whole new set of specific disorders, many of which can now be understood on a molecular level, were reported by various investigators. Various aspects of myogenesis—both in normal and abnormal cells, and during development and in regeneration—were discussed, as were the interaction of nerve and muscle and the biochemistry related to those interactions.

Experiments of nature—in which individuals with muscular dystrophy have also been growth-hormone-deficient or have had denervation, as by polio, but have not developed the usual muscle deterioration—indicate that many environmental factors can affect genetically determined muscle

decreased clearance of free cortisol, and it is presumed that there is increased secretion of corticotropin-releasing factor (CRF). Growth hormone is increased, but somatomedin-C (IGF-I) levels are decreased; this may conserve nitrogen. There is increased sensitivity to insulin, and norepinephrine secretion is reduced. Vasopressin also appears to be reduced and this may cause difficulty in handling water loads.

Consequences of the amenorrhea induced by starvation may be osteoporosis, stress fractures, and aseptic hip necrosis. All of these conditions are much more common in patients with anorexia than in normal females. Osteoporosis may result from scoliosis, but scoliosis may actually precede anorexia, an interesting observation.

Dr. George F. Koob of the Scripps Clinic and Research Foundation in La Jolla, California, discussed behavioral and endocrine effects of CRF on the central nervous system (CNS). CRF is a potent stimulus for both adrenocorticotropic hormone (ACTH) and beta-endorphin release. It has also been shown to increase CNS activity in a manner much like that of caffeine, and it potentiates the acoustic startle response. CRF also affects the limbic system, with its primary effects on learning and behavioral pathology, aggression, and changes in sexual behavior.

Another presentation at the symposium dealt with the pathophysiology of hypothalamic-pituitary-adrenal dysfunction in depression and anorexia nervosa. Dr. Philip W. Gold of the National Institute of Mental Health of the National Institutes of Health in Bethesda, Maryland, reported that hypothalamic dysfunction has been shown to be present in anorexia nervosa and depression. Moreover, the hypercortisolism present in both disorders appears similar in pathophysiology, but different from that observed in Cushing's disease. Dr. Gold stated that in both depression and anorexia nervosa, there is probable increased secretion of endogenous CRF, attenuated ACTH responses to CRF, and adrenal hyperresponsiveness to ACTH. These abnormalities resolve when the patients gain weight. The hypercortisolism in depression and anorexia nervosa represents a central defect, whereas the hypercortisolism of Cushing's disease is believed to be caused by a defect of excessive ACTH secretion that seems to be localized in the pituitary. Dr. Gold and his co-workers believe that endogenous CRF secretion in patients with depression and anorexia nervosa may be significant in the symptom complexes of these illnesses.

function and deterioration. It appears that the size of muscle cells in Duchenne's muscular dystrophy may be critical in the dystrophic process. Growth hormone deficiency can slow the rate of progression of muscular dystrophy, possibly by limiting the size of the muscle cell. This and other observations give hope that new approaches to symptomatic therapy can be found. Fortunately, new techniques for studying muscle size, composition, and function, such as nuclear magnetic resonance, are beginning to yield clues about normal muscle physiology at the molecular level and about the distribution of abnormalities within the muscle cells.

Many well-known syndromes in which the etiology has not been defined—such as Marfan, Schwartz-Jampel, and Marinesco-Sjögren syndromes—were examined as possible muscular dystrophies.

Perhaps the most exciting recent advance has been the molecular analysis of the Duchenne's muscular dystrophy gene locus. Two approaches have been used: that of "walking" along the X chromosome and the use of DNA from girls with Duchenne's muscular dystrophy who have X-autosome translocations that can be studied on a molecular level. The area of the Duchenne gene is now starting to be "peppered" with probes that allow prenatal diagnosis and carrier detection. It is now considered likely that the gene locus for Becker's muscular dystrophy is either within or very close to that for Duchenne's muscular dystrophy. Linkage analysis of other myopathies and muscle problems is improving as well. For example, the linkage of myotonic dystrophy, by using more closely linked genes, now enables much more accurate prenatal diagnosis and premorbid recognition.

In general, the conference was exciting and stimulating, because it encouraged interaction between basic research scientists and clinicians. It is exciting to see how much progress has been made in an area in which new findings and techniques can be rapidly applied to clinical conditions. We look forward to seeing this same approach being used in future March of Dimes conferences to elucidate other organ systems. In this way, birth defects and genetic diseases will be further delineated.

MEETING CALENDAR

January 25-28 34th Postgraduate Course, American Diabetes Association. Marriott's Orlando, Florida. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22320 (800-232-3472)

March 15-24 International Postgraduate Course in Endocrinology. Siena and Assisi, Italy. Contact: Loretta Giacoletto, Washington University School of Medicine, P.O. Box 8063, 600 South Euclid Street, St. Louis, MO 63110 (800-352-9862)

March 23-27 14th Training Course on Hormonal Assay Techniques. Bethesda, Maryland. Contact: Nettie Karpin, Executive Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 28014 (301-530-9660)

April 27-30 Annual Meeting of the American Pediatric Society/Society for Pediatric Research. Disneyland Hotel, Anaheim, California. Contact: Debbie Wogenrich, Department of Pediatrics, University of New Mexico, Albuquerque, NM 87131 (505-277-6628)

May 1 Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Disneyland Hotel, Anaheim, California. Contact: Dr. Gilbert August, Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue NW, Washington, DC 20010 (202-745-2121)

May 8-14 Spring Session, American Academy of Pediatrics. Ramada Renaissance, San Francisco, California. Contact: Neal Baker, Department of Education, American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016)

June 4-12 47th Annual Meeting and Scientific Sessions, American Diabetes Association. Hyatt Regency Hotel, Indianapolis, Indiana. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22320 (800-232-3472)

June 10-12 69th Annual Meeting, The Endocrine Society. Indianapolis Convention Center, Indianapolis, Indiana. Contact: Nettie Karpin, Executive Director, The Endocrine Society, 9650 Rockville Pike. Bethesda, MD 20814 (301-530-9660)

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